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THE VALUE OF BEEF PROTEIN AS A SUPPLEMENT TO THE PROTEINS IN CERTAIN VEGETABLE PRODUCTS[1]

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INTRODUCTION

The nutritive value of a protein when fed as the single source of nitrogen in the diet is not necessarily the same as when it is fed in combination with other proteins. If a protein supplies adequate proportions of all the amino acids required by the body, the nutritive value of such a protein will not be enhanced by combining it with other proteins; but, on the other hand, if a protein is deficient in one or more of the essential amino acids, its nutritive value will be greatly increased by feeding it in combination with other proteins which furnish an excess of these acids. To combine proteins in the dietary in such a way as to supply a mixture of high biological value is a matter of very practical importance.

Most meat proteins have a high biological value, but the proteins in the vegetable products which constitute a large part of our dietary are more or less deficient in the essential amino acids. The purpose of the investigation herein reported was to determine the extent to which the protein in beef supplements that in wheat, bolted wheat flour, corn meal, oatmeal, rice, navy beans, and potatoes.

PREVIOUS INVESTIGATIONS

Hoagland and Snider (*1*, *2*)[2] studied the nutritive value of the protein in the lean meat and in most of the important edible organs of cattle, calves, sheep, and hogs by means of quantitative feeding experiments with young albino rats. They found that, with the exception of a few products of minor importance, the meats and meat products contained protein of high biological value. Previous investigations concerning the nutritive value of meat proteins are discussed in those papers.

The nutritive value of the protein in wheat and in bolted wheat flour has been determined by Osborne and Mendel (*8*, *p. 600–601*). They state:

> The quantity of protein furnished by the *entire* wheat kernel which is necessary for continued maintenance of adult rats is greater than that required when proteins of milk or of various other food products are fed. For the normal growth of the young this difference is much more pronounced.
>
> For maintaining adults the proteins of the endosperm are adequate; they are inadequate for growth. Additions of meat, milk, or eggs to wheat flour so greatly

[1] Received for publication Aug. 21, 1926; issued April, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 307.

enhance the value of the protein for growth that a great economy in consumption of protein results. Under the ordinary human dietary conditions the proteins of wheat are advantageously employed.

In another paper the same authors (*9, p. 293*) report the results of a study of the nutritive value of the proteins of the barley, oat, rye, and wheat kernels. They conclude that "until a far greater refinement of the method is secured it would appear, on the whole, that the proteins of the four cereals studied are not widely different in their efficiency in promoting growth."

McCollum, Simmonds, and Parsons (*4*) report that the proteins of kidney, liver, and muscle are remarkably valuable for supplementing the proteins of some of the cereals, but that they are less satisfactory as supplements of the legume proteins. They state also that the proteins of kidney, liver, or muscle are more valuable for transformation into body tissue when combined with cereal proteins than when each is fed as the sole source of amino acids in the diet.

McCollum and Simmonds (*3, p. 129, 131, 133, 135*) place a high value on the proteins of the entire wheat kernel. They state that wheat protein is slightly more effective in promoting growth than the same amount of protein from milk or eggs. However, they also state that feeding tests in which wheat was the sole source of protein in the diet were not uniformly successful and that apparently some wheats are superior to others. Bolted wheat flour is reported to contain protein of poor quality. Maize, when fed as the sole source of protein in the diet, is considered to have a somewhat lower nutritive value than wheat, and rice is reported to contain proteins of low nutritive value.

According to Sherman (*11, p. 247, 231*) the proteins of meat adequately supplement those of grains in nutrition. Wheat, maize, and oat proteins were found to have practically the same value in human nutrition.

Mitchell (*5*) determined the biological value of the protein in a number of food products by means of feeding tests with rats. When the rats were fed at the 5 per cent level the following biological values were obtained: 93.4 for milk, 72 for corn, 78.6 for oats, 86.1 for rice, and 68.5 for potatoes. When the rats were fed at the 10 per cent level, the values were 59.6 for corn, 64.9 for oats, 66.7 for potatoes, and 38.4 for navy beans. These figures refer to digestible protein.

Mitchell and Carman (*6*) report the biological values obtained for the protein in certain food products when fed at an 8 per cent level of intake. Whole wheat had a value of 67, pork 74, and eggs 93.

In a recent publication Mitchell and Carman (*7*) report the biological values obtained for the nitrogen in mixtures of patent white flour and certain animal products. When two parts of white-flour protein were fed in combination with one part of protein from beef, veal, eggs, or milk, the biological value of the protein in the white flour was very materially enhanced. The protein in the mixture of beef and white flour or veal and white flour had as high a biological value as did either beef or veal alone, whereas white flour alone had a much lower value. The supplemental relationship between the proteins in the products tested was found to be greatest between white flour and beef and least between white flour and egg albumin. No distinct difference between the other animal products appeared to exist.

EXPERIMENTAL WORK

PROCEDURE

The methods employed in this investigation are essentially the same as those followed by the writers in previous studies of the nutritive value of the protein in various animal tissues (*1*, *2*). Each ration was fed to six young male albino rats for a period of 60 days. The rats were housed in individual cages and an accurate record was kept of the feed consumed. The rats weighed approximately 40 grams each at the beginning of the experiment and they were weighed twice weekly during the tests.

Each ration was made up to contain 10 per cent protein [3] (Nx6.25), exclusive of that in the vitamin B; 10 per cent fat, of which 2 per cent was cod-liver oil as a source of vitamins A and D; 4 per cent ash mixture; 2 per cent vitamin B from yeast; and the rest cassava starch, which has been found to be practically free from nitrogen. The vitamin B product contained approximately 50 per cent protein so that about 1 per cent of crude protein from this source was added to the ration.

DESCRIPTION OF PRODUCTS TESTED

The beef consisted of round steak from high-grade beef. The muscle was trimmed as free from fatty and connective tissues as practicable, ground, mixed with water and toluol, and dried in a current of air at 60° C. Several lots of beef were used in the experiments.

Three lots of yellow corn meal, two lots each of hard spring wheat, northwestern fancy patent flour, oatmeal, and potatoes, and one lot each of ordinary commercial rice and navy beans were used. The navy beans were soaked overnight, boiled until soft, ground, and dried in a current of air. The potatoes were peeled, boiled until thoroughly cooked, ground, and dried in a current of air. All the products named above, except the flour and corn meal, were ground to fine condition before they were mixed in the rations.

RESULTS OF EXPERIMENTS WITH RATIONS CONTAINING 10 PER CENT OF ANIMAL OR VEGETABLE PROTEIN

In Table 1 are reported the results of the 30-day experiments with rations containing 10 per cent each of protein from beef, pork, lamb, wheat, bolted wheat flour, oatmeal, and navy beans, respectively. Since there are greater or lesser variations in the results obtained from the individual rats fed a single ration, judgment of the apparent relative nutritive value of the protein in the different products should be based upon the average data for each group of rats.

[3] The writers have found that 10 per cent of beef protein in a ration containing 10 per cent of fat is somewhat less protein than is required for optimal growth in young rats during a period of 60 days. Five per cent of beef protein is adequate only for maintenance.

TABLE 1.—*Comparative nutritive values of animal and vegetable proteins when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Ox muscle (beef)	422 A	32	48	77	279	27.9	3.6	0.36	0.28	2.76
Do	422 B	32	51	113	359	35.9	3.2	.32	.31	3.15
Do	422 D	32	49	98	302	30.2	3.1	.31	.32	3.24
Do	588 D	26	38	92	268	26.8	2.9	.29	.34	3.43
Average		31	47	95	302	30.2	3.2	.32	.31	3.15
Hog muscle (pork)	384 C	24	48	84	281	28.1	3.3	.33	.30	2.99
Do	384 D	24	46	86	288	28.8	3.3	.33	.30	2.99
Do	570 A	24	40	66	250	25.0	3.8	.38	.26	2.64
Do	570 B	24	40	98	305	30.5	3.1	.31	.32	3.21
Average		24	44	84	281	28.1	3.4	.34	.30	2.96
Sheep muscle (lamb)	444 B	25	39	87	289	28.9	3.3	.33	.30	3.01
Do	571 A	24	40	84	266	26.6	3.2	.32	.32	3.16
Do	571 B	24	42	93	274	27.4	2.9	.29	.34	3.39
Do	571 C	24	41	77	255	26.5	3.4	.34	.29	2.91
Average		24	41	85	274	27.4	3.2	.32	.31	3.12
Wheat	720 A	26	45	39	180	18.0	4.6	.46	.22	2.17
Do	720 B	26	44	39	171	17.1	4.4	.44	.23	2.28
Do	720 C	26	45	33	171	17.1	5.2	.52	.19	1.93
Do	720 D	22	43	37	200	20.0	5.4	.54	.19	1.85
Do	720 E	22	41	41	186	18.6	4.5	.45	.22	2.20
Do	720 F	23	41	37	182	18.2	4.9	.49	.20	2.03
Average		24	43	38	182	18.2	4.8	.48	.21	2.08
Wheat flour (patent)	722 A	30	40	17	154	15.4	9.0	.90	.11	1.10
Do	722 B	26	41	27	178	17.8	6.6	.66	.15	1.52
Do	722 C	24	38	18	139	13.9	7.7	.77	.13	1.30
Do	722 D	24	40	21	180	18.0	8.6	.86	.12	1.17
Do	722 E	23	39	15	137	13.7	9.1	.91	.11	1.09
Do	722 F	23	40	19	139	16.3	7.3	.73	.14	1.37
Average		25	40	20	155	15.5	8.1	.81	.13	1.27
Oatmeal	710 A	28	44	46	200	20.0	4.3	.43	.23	2.30
Do	710 B	28	41	45	171	17.1	3.8	.38	.26	2.63
Do	710 C	25	43	55	230	23.0	4.2	.42	.24	2.39
Do	710 D	25	38	29	183	18.3	6.3	.63	.16	1.58
Do	710 E	28	48	31	179	17.9	5.8	.58	.17	1.73
Do	710 F	27	40	43	170	17.0	4.0	.40	.25	2.53
Average		27	42	42	189	18.9	4.7	.47	.22	2.19
Navy beans	752 A	23	38	32	191	19.1	6.0	.60	.17	1.67
Do	752 B	23	39	35	211	21.1	6.0	.60	.17	1.66
Do	752 C	23	38	10	135	13.5	13.5	1.35	.07	.74
Do	752 D	23	40	24	181	18.1	7.5	.75	.13	1.33
Do	752 E	23	40	39	211	21.1	5.4	.54	.18	1.85
Do	752 F	28	44	31	188	18.8	6.1	.61	.16	1.65
Average		24	40	29	186	18.6	7.4	.74	.15	1.48

The data in Table 1 show very clearly that the protein in beef, pork, and lamb was very much more efficient for maintenance and growth of albino rats when fed at the 10 per cent level for 30 days than was the protein in wheat, bolted wheat flour, oatmeal, or navy beans. The protein in wheat and in oatmeal had approximately the same nutritive value, followed in turn by that in navy beans and bolted wheat flour. The gain in weight per gram of protein consumed ranged from 2.96 to 3.15 grams for the rats fed the meat proteins as compared with 1.23 to 2.19 grams for those fed the vegetable proteins. The rats fed meat proteins made an average gain in weight of 88 grams during the 30-day test, whereas the best average gain made by those fed any one of the vegetable proteins was only 42 grams.

In Table 2 are reported the results of the 60-day feeding tests with rations containing 10 per cent each of meat or vegetable protein. Again the meat proteins were very much more efficient in promoting growth than the vegetable proteins. Among the vegetable products oatmeal seemed to be a slightly better source of protein than wheat, while bolted wheat flour and navy beans were considerably less satisfactory. The rats getting meat protein made an average gain in weight of 153 grams during the 60 days of the experiment, whereas those fed vegetable proteins made gains ranging from 41 grams for bolted wheat flour to 82 grams for oatmeal. The rats fed meat protein gained, on an average, 2.5 grams in weight for each gram of protein consumed, but the rats fed vegetable proteins made gains ranging only from 1.16 grams for bolted wheat flour to 1.89 grams for oatmeal.

TABLE 2.—*Comparative nutritive values of animal and vegetable proteins when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Ox muscle (beef)	422 A	32	48	146	603	60.3	4.1	0.41	0.24	2.42
Do	422 B	32	51	189	776	77.6	4.1	.41	.24	2.44
Do	422 D	32	49	172	687	68.7	4.0	.40	.25	2.50
Do	588 D	26	38	171	601	60.1	3.5	.35	.28	2.85
Average		31	47	170	667	66.7	3.9	.39	.25	2.55
Hog muscle (pork)	384 C	24	48	127	582	58.2	4.6	.46	.22	2.18
Do	384 D	24	46	147	550	55.0	3.7	.37	.27	2.67
Do	570 A	24	40	111	485	48.5	4.4	.44	.23	2.29
Do	570 B	24	40	163	600	60.0	3.7	.37	.27	2.72
Average		24	44	137	554	55.4	4.1	.41	.25	2.46
Sheep muscle (lamb)	444 B	25	39	152	656	65.6	4.3	.43	.23	2.32
Do	571 A	24	40	157	596	59.6	3.8	.38	.26	2.63
Do	571 B	24	42	162	616	61.6	3.8	.38	.26	2.63
Do	571 C	24	41	135	575	57.5	4.3	.43	.23	2.35
Average		24	41	152	611	61.1	4.1	.41	.25	2.48
Wheat	720 A	26	45	81	462	46.2	5.7	.57	.18	1.75
Do	720 B	26	44	73	418	41.8	5.7	.57	.17	1.75
Do	720 C	26	45	67	418	41.8	6.2	.62	.16	1.60
Do	720 D	22	43	74	479	47.9	6.5	.65	.15	1.54
Do	720 E	22	41	69	431	43.1	6.2	.62	.16	1.60
Do	720 F	23	41	60	476	47.6	7.9	.79	.13	1.26
Average		24	43	71	447	44.7	6.4	.64	.16	1.58
Wheat flour (patent)	722 A	30	40	39	356	35.6	9.1	.91	.11	1.10
Do	722 B	26	41	51	398	39.8	7.8	.78	.13	1.28
Do	722 C	24	38	43	328	32.8	7.6	.76	.13	1.31
Do	722 D	24	40	46	394	39.4	8.6	.86	.12	1.17
Do	722 E	23	39	28	285	28.5	10.2	1.02	.10	.98
Do	722 F	23	40	39	351	35.1	9.0	.90	.11	1.11
Average		25	40	41	352	35.2	8.7	.87	.12	1.16
Oatmeal	710 A	28	44	78	442	44.2	5.7	.57	.18	1.76
Do	710 B	28	41	91	412	41.2	4.5	.45	.22	2.21
Do	710 C	25	43	119	559	55.9	4.7	.47	.21	2.13
Do	710 D	25	38	34	315	31.5	9.3	.93	.11	1.08
Do	710 E	28	48	81	423	42.3	5.2	.52	.19	1.91
Do	710 F	27	40	88	395	39.5	4.5	.45	.22	2.23
Average		27	42	82	424	42.4	5.7	.57	.19	1.89
Navy beans	752 A	23	38	45	430	43.0	9.6	.96	.10	1.05
Do	752 B	23	39	67	462	46.2	6.9	.69	.15	1.45
Do	752 C	23	38	35	293	29.3	8.4	.84	.12	1.19
Do	752 D	23	40	57	399	39.9	7.0	.70	.14	1.43
Do	752 E	23	40	62	420	42.0	6.8	.68	.15	1.48
Do	752 F	28	44	50	376	37.6	7.5	.75	.13	1.33
Average		24	40	53	397	39.7	7.7	.77	.13	1.32

RESULTS OF EXPERIMENTS WITH RATIONS CONTAINING 5 PER CENT EACH OF ANIMAL AND OF VEGETABLE PROTEIN

In Table 3 are reported the results of the 30-day experiments with rations containing each 5 per cent of beef protein and 5 per cent of protein from wheat, bolted wheat flour, oatmeal, corn meal, rice, navy beans, and potatoes, respectively.

TABLE 3.—*Comparative nutritive values of mixtures of equal parts of beef and vegetable proteins when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	721 A	24	44	108	305	30.5	2.8	0.28	0.35	3.54
Do	721 B	28	45	97	289	28.9	3.0	.30	.34	3.36
Do	721 C	27	39	94	285	28.5	3.0	.30	.33	3.30
Do	721 D	28	39	80	256	25.6	3.2	.32	.31	3.12
Do	721 E	28	41	68	236	23.6	3.5	.35	.29	2.88
Do	721 F	28	43	92	294	29.4	3.2	.32	.31	3.13
Average		27	42	90	278	27.8	3.1	.31	.32	3.22
Beef and wheat flour (patent)	723 A	23	39	69	229	22.9	3.3	.33	.30	3.01
Do	723 B	23	39	67	239	23.9	3.6	.36	.28	2.80
Do	723 C	24	39	78	247	24.7	3.2	.32	.32	3.16
Do	723 D	28	41	76	259	25.9	3.4	.34	.29	2.93
Do	723 E	24	40	64	234	23.4	3.7	.37	.27	2.74
Do	723 F	24	37	79	259	25.9	3.3	.33	.31	3.05
Average		24	39	72	245	24.5	3.4	.34	.30	2.95
Beef and corn meal	716 A	26	38	96	266	26.6	2.8	.28	.36	3.61
Do	716 B	26	39	66	231	23.1	3.5	.35	.29	2.86
Do	716 C	30	39	75	246	24.6	3.3	.33	.30	3.04
Do	716 D	30	44	71	254	25.4	3.6	.36	.28	2.80
Do	716 E	24	44	123	340	34.0	2.8	.28	.36	3.62
Do	716 F	24	44	96	316	31.6	3.3	.33	.30	3.04
Average		27	41	88	276	27.6	3.2	.32	.32	3.16
Beef and oatmeal	711 A	26	43	65	214	21.4	3.3	.33	.30	3.04
Do	711 B	26	41	84	247	24.7	2.9	.29	.34	3.40
Do	711 C	26	42	92	325	32.5	3.5	.35	.28	2.83
Do	711 D	26	38	74	245	24.5	3.3	.33	.30	3.02
Do	711 E	26	44	94	297	29.7	3.2	.32	.32	3.16
Do	711 F	27	41	95	286	28.6	3.0	.30	.33	3.32
Average		26	42	84	269	26.9	3.2	.32	.31	3.13
Beef and rice	749 A	27	39	110	310	31.0	2.8	.28	.35	3.55
Do	749 B	25	44	120	362	36.2	3.0	.30	.33	3.31
Do	749 C	25	46	110	343	34.3	3.1	.31	.32	3.21
Do	749 D	25	44	120	352	35.2	2.9	.29	.34	3.41
Do	749 E	25	41	104	330	33.0	3.2	.32	.32	3.15
Do	749 F	28	39	115	338	33.8	2.9	.29	.34	3.40
Average		26	42	113	339	33.9	3.0	.30	.33	3.34
Beef and navy beans	753 A	25	39	44	196	19.6	4.5	.45	.22	2.24
Do	753 B	26	39	49	209	20.9	4.3	.43	.23	2.34
Do	753 C	26	40	59	253	25.3	4.3	.43	.23	2.33
Do	753 D	27	40	49	223	22.3	4.6	.46	.22	2.19
Do	753 E	27	39	59	230	23.0	3.9	.39	.26	2.57
Do	753 F	27	39	43	192	19.2	4.5	.45	.22	2.24
Average		26	39	51	217	21.7	4.4	.44	.23	2.32
Beef and potatoes	766 A	27	45	23	150	15.0	6.5	.65	.15	1.53
Do	766 B	27	45	26	149	14.9	5.7	.57	.17	1.74
Do	766 C	27	43	21	141	14.1	6.7	.67	.15	1.49
Do	766 D	24	42	34	163	16.3	4.8	.48	.21	2.09
Do	766 E	24	43	29	153	15.3	5.3	.53	.19	1.89
Do	766 F	24	41	31	152	15.2	4.9	.49	.20	2.04
Average		26	43	27	151	15.1	5.7	.57	.18	1.80

The results of these experiments indicate that beef protein very greatly enhances the nutritive value of the protein in our four most important food cereals when the two kinds of protein are mixed in equal proportions in the diet. The rations containing wheat, bolted wheat flour, corn meal, oatmeal, and rice, respectively, were approximately of equal value in promoting growth, and this value was about the same as that previously obtained for rations containing the same percentage of meat protein alone. (Table 1.) On the other hand, the rations containing beef and navy beans or potatoes had much lower values.

Among the rats fed beef and cereal rations, the gain in weight per gram of protein consumed during the 30-day test ranged from 2.95 grams for those fed the beef-wheat flour ration to 3.34 grams for those fed the beef-rice ration, as compared with an average gain of 3.08 grams for those fed the rations containing meat protein alone.

In Table 4 are reported the results of the 60-day tests with rations containing equal parts of beef and vegetable proteins. As in the 30-day feeding experiments with the same rations, these tests show that the rations containing equal parts of beef and cereal proteins were practically of the same value in promoting growth in rats as rations containing only meat protein. The rations containing equal parts of beef and bean or potato protein, however, were of much less value, as in the 30-day experiment.

TABLE 4.—*Comparative nutritive values of mixtures of equal parts of beef and vegetable proteins when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	721 A	24	44	173	695	69.5	4.0	0.40	0.25	2.49
Do	721 B	28	45	125	601	60.1	4.8	.48	.21	2.08
Do	721 C	27	39	154	662	66.2	4.3	.43	.23	2.33
Do	721 D	28	39	139	617	61.7	4.4	.44	.23	2.25
Do	721 E	28	41	118	537	53.7	4.6	.46	.22	2.20
Do	721 F	28	43	140	624	62.4	4.5	.45	.22	2.24
Average		27	42	142	623	62.3	4.4	.44	.23	2.27
Beef and wheat flour (patent)	723 A	23	39	162	590	59.0	3.6	.36	.27	2.75
Do	723 B	23	39	154	594	59.4	3.8	.38	.26	2.59
Do	723 C	24	39	155	588	58.8	3.8	.38	.26	2.64
Do	723 D	28	41	119	538	53.8	4.5	.45	.22	2.21
Do	723 E	24	40	143	603	60.3	4.2	.42	.24	2.37
Do	723 F	24	37	158	613	61.3	3.9	.39	.26	2.58
Average		24	39	149	588	58.8	3.9	.38	.25	2.53
Beef and corn meal	716 A	26	38	165	624	62.4	3.8	.38	.26	2.64
Do	716 B	26	39	123	539	53.9	4.4	.44	.23	2.28
Do	716 C	30	39	147	599	59.9	4.1	.41	.25	2.45
Do	716 D	30	44	140	616	61.6	4.4	.44	.23	2.26
Do	716 E	24	44	195	756	75.6	3.9	.39	.26	2.58
Do	716 F	24	44	182	760	76.0	4.2	.42	.24	2.40
Average		27	41	159	649	64.9	4.1	.41	.25	2.44
Beef and oatmeal	711 A	26	43	161	577	57.7	3.6	.36	.28	2.79
Do	711 B	26	41	159	610	61.0	3.8	.38	.26	2.61
Do	711 C	26	42	143	678	67.8	4.7	.47	.21	2.11
Do	711 D	26	38	158	637	63.7	4.0	.40	.25	2.48
Do	711 E	26	44	185	715	71.5	3.9	.39	.26	2.59
Do	711 F	27	41	175	697	69.7	4.0	.40	.25	2.51
Average		26	42	164	652	65.2	4.0	.40	.25	2.52
Beef and rice	749 A	27	39	189	707	70.7	3.7	.37	.27	2.67
Do	749 B	25	44	195	773	77.3	4.0	.40	.25	2.52
Do	749 C	25	46	173	729	72.9	4.2	.42	.24	2.37
Do	749 D	25	44	201	749	74.9	3.7	.37	.27	2.68
Do	749 E	25	41	174	719	71.9	4.1	.41	.24	2.42
Do	749 F	28	39	167	704	70.4	4.2	.42	.24	2.37
Average		26	42	183	730	73.0	4.0	.40	.25	2.50
Beef and navy beans	753 A	25	39	102	501	50.1	4.9	.49	.20	2.04
Do	753 B	26	39	87	447	44.7	5.1	.51	.19	1.95
Do	753 C	26	40	111	563	56.3	5.1	.51	.20	1.97
Do	753 D	27	40	103	497	49.7	4.8	.48	.21	2.07
Do	753 E	27	39	120	563	56.3	4.7	.47	.21	2.13
Do	753 F	27	39	88	441	44.1	5.0	.50	.20	2.00
Average		26	39	102	502	50.2	4.9	.49	.20	2.03
Beef and potatoes	766 A	27	45	57	337	33.7	5.9	.59	.17	1.69
Do	766 B	27	45	65	356	35.6	5.5	.55	.18	1.83
Do	766 C	27	43	56	335	33.5	6.0	.60	.17	1.67
Do	766 D	24	42	88	430	43.0	4.9	.49	.20	2.05
Do	766 E	24	43	72	364	36.4	5.1	.51	.20	1.98
Do	766 F	24	41	63	346	34.6	5.5	.55	.18	1.82
Average		26	43	67	361	36.1	5.5	.55	.18	1.84

RESULTS OF EXPERIMENTS WITH RATIONS CONTAINING 6.67 PER CENT OF VEGETABLE PROTEIN AND 3.33 PER CENT OF ANIMAL PROTEIN

In Table 5 are reported the results of the 30-day experiments with rations containing two parts of vegetable protein and one part of animal protein. In these experiments the rations containing beef and cornmeal or oatmeal induced appreciably greater and more economical growth then the rations containing beef and wheat or wheat flour. The rations containing beef and cornmeal or oatmeal were fully as efficient in promoting growth during a 30-day test as were rations containing a like percentage of beef protein alone. (Table 1.) Rice, on account of its low protein content, could not be used in a ration in sufficient quantity to furnish two parts of protein to one part of beef protein.

TABLE 5.—*Comparative nutritive values of mixtures of one-third beef protein and two-thirds vegetable protein when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	756 A	25	41	83	260	26.0	3.1	0.31	0.32	3.19
Do	756 B	25	42	75	269	26.9	3.6	.36	.28	2.79
Do	756 C	25	40	87	299	29.9	3.4	.34	.29	2.91
Do	756 D	25	45	69	264	26.4	3.8	.38	.26	2.61
Do	756 E	25	45	60	253	25.3	4.2	.42	.24	2.37
Do	756 F	25	43	48	217	21.7	4.5	.45	.22	2.21
Average		25	43	70	260	26.0	3.8	.38	.27	2.68
Beef and wheat flour (patent)	765 A	24	40	55	216	21.6	3.9	.39	.25	2.55
Do	765 B	24	39	65	243	24.3	3.7	.37	.27	2.67
Do	765 C	24	44	72	253	25.3	3.5	.35	.28	2.84
Do	765 D	24	39	56	227	22.7	4.1	.41	.25	2.47
Do	765 E	23	39	78	285	28.5	3.7	.37	.27	2.74
Do	765 F	23	42	77	284	28.4	3.7	.37	.27	2.71
Average		24	41	67	251	25.1	3.8	.38	.27	2.67
Beef protein 4 per cent and cornmeal protein 6 per cent	770 A	28	38	100	338	33.8	3.4	.34	.29	3.96
Do	770 B	28	43	153	452	45.2	3.0	.30	.34	3.38
Do	770 C	25	41	107	364	36.4	3.4	.34	.29	2.94
Do	770 D	25	38	66	269	26.9	4.1	.41	.25	2.45
Do	770 E	25	39	87	311	31.1	3.6	.36	.28	2.80
Do	770 F	25	40	105	339	33.9	3.2	.32	.31	3.10
Average		26	40	103	346	34.6	3.5	.35	.29	2.94
Beef and oatmeal	771 A	24	40	114	321	32.1	2.8	.28	.36	3.55
Do	771 B	24	42	92	298	29.8	3.2	.32	.31	3.09
Do	771 C	27	46	95	304	30.4	3.2	.32	.31	3.12
Do	771 D	28	40	101	275	27.5	2.7	.27	.37	3.67
Do	771 E	28	42	68	233	23.3	3.4	.34	.29	2.92
Do	771 F	25	41	79	252	25.2	3.2	.32	.31	3.13
Average		26	42	92	281	28.1	3.1	.31	.33	3.25

In Table 6 are given the results of the 60-day experiments with the rations used in the preceding test. The rations containing beef and wheat flour, corn meal, or oatmeal induced approximately the same growth as rations containing the same percentage of meat protein alone. (Table 2.) The beef and wheat ration, however, was of slightly lower value.

TABLE 6.—*Comparative nutritive values of mixtures of one-third beef protein and two-thirds vegetable protein when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	756 A	25	41	154	622	62.2	4.0	0.40	0.25	2.48
Do	756 B	25	42	154	641	64.1	4.2	.42	.24	2.40
Do	756 C	25	40	161	688	68.8	4.3	.43	.23	2.34
Do	756 D	25	45	149	632	63.2	4.2	.42	.24	2.36
Do	756 E	25	45	119	575	57.5	4.8	.48	.21	2.07
Do	756 F	25	43	89	480	48.0	5.4	.54	.19	1.85
Average		25	43	138	606	60.6	4.5	.45	.23	2.25
Beef and wheat flour (patent)	765 A	24	40	131	516	51.6	3.9	.39	.25	2.54
Do	765 B	24	39	147	597	59.7	4.1	.41	.25	2.46
Do	765 C	24	44	155	604	60.4	3.9	.39	.26	2.57
Do	765 D	24	39	120	503	50.3	4.2	.42	.24	2.39
Do	765 E	23	39	166	688	68.8	4.1	.41	.24	2.41
Do	765 F	23	42	177	684	68.4	3.9	.39	.26	2.59
Average		24	41	149	599	59.9	4.0	.40	.25	2.49
Beef protein 4 per cent and cornmeal protein 6 per cent	770 A	28	38	189	762	76.2	4.0	.40	.25	2.48
Do	770 B	28	43	273	952	95.2	3.5	.35	.29	2.87
Do	770 C	25	41	182	721	72.1	3.9	.39	.25	2.52
Do	770 D	25	38	146	565	56.5	3.9	.39	.26	2.58
Do	770 E	25	39	168	726	72.6	4.3	.43	.23	2.31
Do	770 F	25	40	193	742	74.2	3.8	.38	.26	2.60
Average		26	40	192	745	74.5	3.9	.39	.26	2.56
Beef and oatmeal	771 A	24	40	201	710	71.0	3.5	.35	.28	2.83
Do	771 B	24	42	166	634	63.4	3.8	.38	.26	2.62
Do	771 C	27	46	188	692	69.2	3.7	.37	.27	2.72
Do	771 D	28	40	194	648	64.8	3.3	.33	.30	2.99
Do	771 E	28	42	130	555	55.5	4.3	.43	.23	2.34
Do	771 F	25	41	126	558	55.8	4.4	.44	.23	2.26
Average		26	42	168	633	63.3	3.8	.38	.26	2.63

DISCUSSION AND SUMMARY

In this paper are reported the results of a series of quantitative feeding experiments with young male albino rats to determine the value of the protein in beef as a supplement to that in wheat, bolted wheat flour, corn meal, oatmeal, rice, navy beans, and potatoes. Each ration contained 10 per cent of protein exclusive of approximately 1 per cent in vitamin B from yeast.

When each product was the only source of protein in the diet, beef had a much higher value for stimulating growth than any of the vegetable products; wheat and oatmeal had approximately the same value; navy beans and bolted wheat flour had considerably lower values.

When beef protein was mixed in equal proportions with vegetable protein, the rations containing the following vegetable products had approximately the same efficiency in promoting growth, viz, wheat, bolted wheat flour, corn meal, oatmeal, and rice. Each of these mixtures also had approximately the same value for promoting growth as a ration containing the same percentage of beef protein

alone. The rations containing equal parts of beef and navy-bean or potato protein had much lower values.

When one part of beef protein was mixed with two parts of vegetable protein, the rations containing the following vegetable products had approximately the same value for inducing growth, viz, wheat, bolted wheat flour, corn meal, and oatmeal. Each of these mixtures also had approximately the same value for inducing growth as a ration containing the same percentage of beef protein alone.

It is interesting to note that, although the entire wheat kernel furnished protein considerably more efficient in promoting growth than did bolted wheat flour, when each product was the only source of protein in the diet, this difference was no longer apparent if either wheat or bolted wheat flour was fed in combination with beef protein. These facts indicate that bolted wheat flour is probably as good a source of protein in the human dietary as flour made from the entire wheat kernel, provided a reasonable amount of beef or other animal protein of high biological value is included in the dietary.

The high supplemental value which the protein in beef, as well as in many other animal products, has for the protein in the cereals is of great practical importance in human nutrition. According to Pearl (*10, p. 226*), the average percentage amounts of protein consumed in this country during the period 1911–12 to 1916–17 were obtained from the following sources: Grains, 36; meats, 26; dairy products, 20; poultry and eggs, 7; vegetables, 6; fish, 2; miscellaneous, 3. Of these, meats, dairy products, poultry, eggs, and fish, making a total of 55 per cent, contain protein of high biological value; the other products, chiefly grains and their milled products, contain protein of relatively low biological value. This classification is based upon experiments with small animals in which each product was the only source of protein in the diet, and it does not necessarily indicate the nutritive value of the protein in these foods when consumed in a mixed diet. Since the protein in beef, as well as in many other animal products, greatly enhances the value of the protein in the grains, it is highly probable that the cereal proteins will be utilized very efficiently when they are included in a mixed diet containing meat or other animal proteins.

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THE NITROGEN COMPOUNDS OF THE RICE KERNEL AS COMPARED WITH THOSE OF OTHER CEREALS[1]

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INTRODUCTION

A more complete knowledge of the constituents of rice and of their significance is desirable, not only from a physiological and biochemical standpoint, but also from that of utility. After polypeptides and free amino acids had been shown to occur in the ungerminated kernel of wheat (*Triticum vulgare*) (*10*),[3] oats (*Avena sativa*) (*8*), corn (*Zea mays*) (*9*), and rye (*Secale cereale*) (*11*), it was reasonable to assume that those compounds were likely to be present also in the ungerminated kernel of rice (*Oryza sativa*). The fact that rice, however, is the chief food of more than half of the world's population and has maintained that position through thousands of years, coupled with the circumstance that polypeptides have never been reported to occur in the rice kernel, made it desirable to ascertain whether polypeptides and free amino acids occur in the different varieties of rice, and if so, in what proportions. The desire to clear up these points was augmented by the fact that ordinarily the results found in the literature are not related to definite varieties, and that different cereals have dissimilar characteristics. Thus, wheat is characterized by the high nitrogen and protein content of its kernel and is the only cereal the flour of which forms a coherent gluten (*16*). The rice kernel contains little nitrogen and protein, but it is distinguished by its high digestibility (*14*). In the corn kernel is included a considerable proportion of fat, and in the rye kernel a not inconsiderable quantity of a gummy substance (*16*). Other characteristics of these cereals will be mentioned later on.

REVIEW OF LITERATURE

The literature contains many records of analyses of the constituents of rice, a few of which will be reviewed. The analytical results of Wise and Broomell (*24, p. 27*) show that Honduras rice (fancy head, uncoated) contains 0.38 per cent ash, 0.19 per cent ether extract, 0.24 per cent crude fiber, 8.74 per cent protein, and 2.20 per cent pentosans, calculated to the moisture-free basis. Rosenheim and Kajiura (*17*) state that the proteins in table rice make up about 7 per cent of the grain as used for food. They class the proteins as rice globulin, rice albumin, and oryzenin. These results were confirmed by Suzuki, Yoshimura, and Fuji (*23*), who state that bran-free rice contains 1.200 per cent of total nitrogen, 1.165 per cent of protein nitrogen, and 0.035 per cent of nonprotein nitro-

[1] Received for publication Sept. 2, 1926; issued April, 1927.
[2] Credit is due J. G. Wangler, formerly junior chemist of the Office of Plant Geography and Physiology, Bureau of Plant Industry, for assistance in doing part of the analytical work.
[3] Reference is made by number (italic) to "Literature cited," p. 324.

gen. Osborne, Van Slyke, Leavenworth, and Vinograd (*14*) have estimated the distribution of nitrogen among the hydrolytic products of oryzenin, the chief protein of the endosperm of rice, and have come to the conclusion that in its general amino-acid make-up it resembles the proteins of animal tissues in a higher degree than do the proteins of wheat or maize. This, in their opinion, explains the extensive use of rice as an exclusive diet throughout the Orient in spite of its low protein content.

From this reveiw it will be seen that these papers contain chiefly data as to the quantity of total nitrogen, protein nitrogen, and nonprotein nitrogen in the rice kernel; and these papers are fairly characteristic of the rest of the literature on rice. No information could be found in regard to the nature of the nonprotein nitrogen, to say nothing of polypeptides, which are not even mentioned in the literature.

RICE VARIETIES EMPLOYED

Four commercially known varieties of rice were used for the experiments recorded in this paper. Wataribune is a long-established commercial variety. It was grown for the first time in this country at Webster, Tex., in 1908, by a Japanese farmer who imported the seed from Japan. It is a rice of high-yielding capacity and is the principal variety cultivated in California. It belongs to the short-grain rices, which have small stalks, narrow leaves, and short kernels (*2*, *12*).

Blue Rose is also a long-established commercial variety, the result of a selection made by Wright from an unknown variety found by Shoemaker in 1907 in a field of Japanese rice east of Jennings, La. Plants of this unknown variety were given by Shoemaker to Wright, who isolated a strain which was later offered for sale under the name Blue Rose. It is the leading variety of medium-grain rice and is grown chiefly in the Southern States (*2*, *12*, *15*).

Another long-known commercial variety is Honduras, which is a long-grain rice, imported from Honduras into Louisiana probably as early as 1890. It is probably a strain of the Creole variety, which is extensively raised in Morelos, Mexico. This rice is widely known and valued for its excellent cooking quality (*2*, *15*).

Fortuna, which is a new variety, is a pure-line selection from the Pa Chiam variety, obtained in 1905 by the United States Department of Agriculture from the Department of Agriculture of Formosa. The selection was made by C. E. Chambliss and J. M. Jenkins in 1911 at the Rice Experiment Station, Crowley, La. (*2*, *3*).

Only brown rice, i. e., seed rice, from which the husk had been removed, was used in the experiments here described. The rice samples were first carefully freed from weeds (red rice), seed rice, shriveled kernels, punctured rice (damaged by insects), chaff, and all foreign matter. They were then dried in an electric oven at about 55° C. for from two to three days, ground, passed through a 40-mesh sieve, and put into sealed jars ready for use.

METHODS AND RESULTS

Some general information with regard to the four rice varieties investigated will be found in Table 1. This investigation was begun in 1923, but owing to pressure of other work it had to be discontinued, and was resumed in January, 1926. For this reason

the data obtained from samples of the 1923 crop and from samples of the 1925 crop are given separately.

Table 1 shows that all the rice samples of 1925 have a lower moisture content than the corresponding samples of 1923. This may be due to the fact that 1925 was a drier season, or to the fact that the climatic conditions at the time of harvest in 1925 were drier than in 1923.

TABLE 1.—*Percentage of moisture, total nitrogen, protein nitrogen, nonprotein nitrogen, and ash in brown rice*

Year grown and variety	Where grown	Moisture in air-dried rice	Total nitrogen in oven-dried rice	Protein nitrogen in—		Nonprotein nitrogen in—		Ash in—	
				Oven-dried rice	Terms of total nitrogen of oven-dried rice	Oven-dried rice	Terms of total nitrogen of oven-dried rice	Air-dried rice	Oven-dried rice
1923		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct*	*P. ct.*
Wataribune, C. I. 1561.	Sacramento Valley, Calif.	8.10	1.19					1.30	1.39
		7.93	1.19					1.29	1.39
		8.01	1.17						
		8.10							
Average		8.04	1.18					1.30	1.39
Blue Rose, C. I. 1962.	Stuttgart, Ark.[a]	10.41	1.29					1.24	1.35
		10.27	1.29					1.25	1.36
		10.23	1.29						
		10.15	1.33						
Average		10.27	1.30					1.25	1.36
Blue Rose, C. I. 1962.	Rice Experiment Station, Crowley, La.	11.05	1.39					1.20	1.31
		10.96	1.38					1.19	1.30
		10.99	1.39						
		10.87							
Average		10.97	1.39					1.20	1.31
Honduras, C. I. 1643.	Rice Experiment Station, Crowley, La.	12.67	1.69					1.22	1.35
		12.49	1.71					1.23	1.36
			1.70						
Average		12.58	1.70					1.23	1.36
Fortuna, C. I. 1344.	Rice Experiment Station, Cowley, La.	11.39	1.53					.88	.96
		11.24	1.53					.89	.97
		11.14	1.53						
			1.52						
Average		11.26	1.53					.89	.97
1925									
Wataribune, C. I. 1561.	Rice Experiment Station, Crowley, La.		1.25	1.18	95.33	[b]	[b]	.86	.93
		8.00	1.24	1.18	95.33	0.062	5.02	.86	.93
		7.90	1.23						
Average		7.95	1.24	1.18	95.33	.062	5.02	.86	.93
Blue Rose, C. I. 1962.	Rice Experiment Station, Crowley, La.	7.44	1.54	1.46	96.92	.060	4.00	1.10	1.19
		7.25	1.51	1.43	94.92			1.11	1.19
			1.48						
Average		7.35	1.51	1.45	95.92	.060	4.00	1.11	1.19
Honduras, C. I. 1643.	Rice Experiment Station, Crowley, La.	8.07	1.38	1.34	97.12	.055	4.02	1.42	1.53
		8.07	1.40					1.45	1.55
			1.37						
Average		8.07	1.38	1.34	97.12	.055	4.02	1.44	1.54
Fortuna, C. I. 1344.	Rice Experiment Station, Crowley, La.	7.55	1.46	1.37	94.17	.093	6.40	1.01	1.09
		7.39	1.45	1.37	94.17	.095	6.52	1.01	1.09
			1.46						
Average		7.47	1.46	1.37	94.17	.094	6.46	1.01	1.09

[a] Grown by the Arkansas Rice Growers' Association.

[b] Lost.

Concerning total nitrogen, it will be seen that in 1923 Honduras had the highest percentage, 1.70; Fortuna the next highest, 1.53; Blue Rose from Louisiana, 1.39; Blue Rose from Arkansas, 1.30; and Wataribune, 1.18; while in 1925 Blue Rose from Louisiana had the most, 1.51; Fortuna had 1.46; Honduras, 1.38; and Wataribune, 1.24. It will also be noticed that Wataribune had practically the same percentage in both seasons, as did Fortuna also; that Honduras dropped from the highest place in 1923 to the third place in 1925, the reverse being the case with Blue Rose from Louisiana.

The quantity of protein nitrogen, calculated on the basis of the oven-dried rice, runs parallel with the quantity of total nitrogen. Thus, for instance, Blue Rose and Wataribune, having the highest and the lowest percentages of total nitrogen, respectively, in 1925, had also the highest and lowest percentages of protein nitrogen. As would be expected, the reverse was in general true of nonprotein nitrogen content.

As Table 1 shows, the ash content tended to be rather uniform. Of the three varieties grown at the Rice Experiment Station at Crowley, La., in 1923, Honduras had the highest ash content, Blue Rose the next highest, and Fortuna the lowest. The same relationship in regard to ash content held good for these three varieties in 1925. However, the Wataribune grown there that year had less ash than any of the three mentioned above, although when grown in California in 1923 it had more than any of them, even more than the Blue Rose grown at Stuttgart, Ark., by the Arkansas Rice Growers' Association, which had a considerably higher ash content than the Blue Rose grown in Louisiana in 1923.

NITROGEN EXTRACTED BY WATER

In order to gain an insight into the nature of the nitrogen compounds in the rice kernel, it is necessary to extract them in such a way that they will not undergo any changes. This is best done by treating the rice flour with boiling hot water, which destroys the enzymes normally present in plant tissues. Preliminary trials were made to ascertain just how much nitrogen is extracted by water. The procedure was as follows: Six flasks, each containing 25 gm. of rice flour, were treated with 500 c. c. of boiling hot ammonia-free water and kept on the water bath for 15 to 30 minutes, after which their contents were centrifuged. The solid residues were treated with boiling hot water once more. The combined extracts were then evaporated in a vacuum, the concentrated substance was centrifuged again, and the supernatant liquids were made up to 500 c. c. Two equal portions were oxidized according to the Kjeldahl method. The results obtained are recorded in Table 2.

An examination of Table 2 shows that water extracted more nitrogen from the Blue Rose variety than it did from the Wataribune rice, and still more from the Honduras variety. This was due partly to the fact that the percentages of total nitrogen in Blue Rose and Honduras are higher than the percentage in Wataribune.

TABLE 2.—*Percentage of nitrogen extracted by water from brown rice*

Sample No.	Variety	Quantity of water extract used	Equivalent of extract in terms of—		Nitrogen extracted by water	
			Air-dry rice	Oven-dried rice	As percentage of oven-dried rice	As percentage of total nitrogen
		C. c.	*Gm.*	*Gm.*		
1	Wataribune	200	60.0	55.40	0.031	2.43
2	do	200	60.0	55.40	.030	2.39
	Average				.031	2.41
3	Blue Rose	200	60.0	55.72	.055	3.64
4	do	200	60.0	55.72	.055	3.63
	Average				.055	3.64
5	Honduras	200	60.0	55.85	.056	4.08
6	do	200	60.0	55.85	.057	4.14
	Average				.057	4.11

ACIDITY OF RICE EXTRACTS

In the course of the experimentation it was noticed that aqueous extracts of rice flour showed acid reaction. In order to ascertain the degree of acidity in the different varieties quantitatively, 25 c. c. portions of the aqueous extract, obtained in the manner outlined above, were diluted with 50 c. c. of distilled water, which was perfectly neutral to phenolphthalein. This dilution was at once titrated with tenth-normal sodium hydroxide, phenolphthalein being used as the indicator. The data are reported in Table 3.

TABLE 3.—*Acidity in rice of different varieties*

Sample No.	Variety	Equivalent of extract used in terms of—		N/10 NaOH used for neutralization
		Air-dry rice	Oven-dried rice	
		Gm.	*Gm.*	*C. c.*
1	Wataribune	7.5	6.925	0.93
2	do	7.5	6.925	.93
3	do	7.5	6.965	.94
	Average			.93
4	Blue Rose	7.5	6.965	1.96
5	do	7.5	6.965	1.92
6	do	7.5	6.925	1.93
	Average			1.94
7	Honduras	7.5	6.981	3.12
8	do	7.5	6.981	3.02
9	do	7.5	6.981	3.07
	Average			3.07

By referring to Table 3 it will be seen that Honduras and Wataribune show the highest and lowest acidity, respectively, while Blue Rose is between the two.

EXTRACTION OF THE VARIOUS NITROGEN COMPOUNDS

Since only about 5 per cent of the total nitrogen of rice is made up of nonnitrogenous compounds and only part of these is extracted by water, it seemed necessary to use a considerable quantity of rice in order to demonstrate the nature and quantity of the nitrogen compounds occurring in the rice kernel. Hence, several series of six flasks were provided, each flask containing 25 gm. of flour and treated with 500 c. c. of boiling hot water from which the ammonia had been removed by long boiling. The flasks were kept on the steam bath for about half an hour. After that their contents were partly filtered on Büchner filters with suction and partly centrifuged. The solid residues were combined and extracted once more in like manner. All of the extracts were then evaporated under reduced pressure, the substance thus concentrated was centrifuged again, and the combined supernatant liquids were finally evaporated to dryness in vacuo. The dry residue of the aqueous extract was then extracted with 85 per cent alcohol in order to get rid of impurities such as starch, proteins, and inorganic salts.

The alcoholic extract obtained was filtered and again evaporated to dryness under diminished pressure. The residual yellow sirup which resulted was dissolved in hot water, filtered, cooled, and made up to 100 c. c. in two 10 c. c. portions, of which the nitrogen was estimated according to the Kjeldahl method, while 10 c. c. was used for qualitative tests.

PHOSPHOTUNGSTIC ACID PRECIPITATE

The remaining 70 c. c. of the solution was made up to 100 c. c. or its multiple, depending on the amount of nitrogen present. To every 100 c. c. of the solution was added 5 gm. of concentrated sulphuric acid and ordinarily 30 c. c. of a phosphotungstic acid solution containing 20 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. After 24 hours the individual precipitates were filtered off on separate filters and washed with a solution containing 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. These were then oxidized according to the Kjeldahl method.

DETERMINATION OF ACID AMIDES

To the combined filtrates and washings from the various phosphotungstic precipitates calcium hydroxide was added to slight acidity, then barium hydroxide was added until distinct alkalinity was indicated. The whole was then saturated with carbon dioxide, boiled, and filtered on a Büchner filter. The cake remaining on the Büchner was extracted for the second time with boiling hot ammonia-free water. When the combined filtrates and washings, which were then concentrated in vacuo, were filtered, they left on the filter white crystals, identified as gypsum.

The filtrate from the gypsum crystals was made up to 200 c. c. In two 10 c. c. portions of this the nitrogen was estimated according to the Kjeldahl method. The 180 c. c. was made up to 200 c. c. and divided into two 100 c. c. portions. Each of these, on receiving 9 c. c. of concentrated hydrochloric acid, was boiled in a glycerin

bath for two hours, a reflux condenser being used. On cooling, each hydrolysate was almost neutralized with sodium hydroxide and distilled with magnesium oxide previously reduced to cream with 100 c. c. of water. The titration of the distillates then gave the ammoniacal nitrogen corresponding to the acid amides present.

HUMIN NITROGEN

The residues in the two Kjeldahl flasks after the ammonia had been driven off were decanted through two Büchners, each of which was provided with a hardened filter. Each Kjeldahl flask was then treated a number of times with boiling hot ammonia-free water and the contents were decanted each time through the Büchners. These were next washed with hot water and sucked perfectly dry. The black substance could then be easily removed from the hardened filters and finally washed down with water quantitatively into 500 c. c. Kjeldahl flasks. The contents of both the Kjeldahl flasks were oxidized in the usual way and distilled, a procedure which yielded the humin nitrogen.

NITROGEN OF AMINO ACIDS

The filtrates and washings from the two magnesium oxide residues were together concentrated on the water bath and made up to 200 c. c. Of this solution 100 c. c. was used for the estimation of the amino nitrogen, while the other 100 c. c. was employed for the determination of the peptide nitrogen, on hydrolysis. Of the first 100 c. c., two portions of 10 c. c. each were Kjeldahlized in order to ascertain the quantity of nitrogen present, 30 c. c. was used for qualitative tests, while in 50 c. c. the amino nitrogen was estimated by Sörensen's method (*6*, *7*, *21*).

PEPTIDE NITROGEN

The work of Fischer (*4*, *p. 23–53*) showed that the polypeptides represent complex compounds in which the amino acids are linked amidelike, and that a complete hydrolysis splits them so that free amino acids result. Hence, the procedure in estimating peptide nitrogen was as follows: To the 100 c. c. of the solution supposed to contain amino acids and polypeptides, concentrated hydrochloric acid was added to a concentration of 20 per cent. The solution was boiled in a glycerin bath under a reflux condenser for 12 hours (the temperature of the bath was about 125° to 127° C.). The hydrolysate was then evaporated on the water bath practically to dryness, taken up with hot water, boiled for a few minutes with carbon black (if necessary), again concentrated on the water bath, and made up to 100 c. c. In two 10 c. c. portions of this the nitrogen was estimated according to the Kjeldahl method; in the 50 c. c. the total amino nitrogen was determined by the formol-titration method, and the residual solution was employed for qualitative tests. Since the amino nitrogen found here represented not only the peptide nitrogen resulting from the hydrolysis but also the free amino nitrogen which was present as such in the solution, it was necessary to subtract this latter nitrogen from the total amino nitrogen found in order to obtain the peptide nitrogen only. The results obtained by the methods outlined are summarized in Table 4.

TABLE 4.—*Distribution of the nonprotein nitrogen in the ungerminated rice kernel (brown rice)*

Variety and year grown	Nitrogen in phosphotungstic precipitate	Nitrogen of acid amides	Humin nitrogen	Nitrogen of amino acids	Peptide nitrogen
	Percentage of oven-dried rice				
Wataribune:					
1925	0.015	0.002	0.003	0.009	0.010
1923		.002	.003	.010	.011
Average	.015	.002	.003	.010	.011
Blue Rose:					
1925	.029	.002	.003	.021	.025
1923		.003	.003	.021	.024
Average	.029	.003	.003	.021	.025
Honduras:					
1925	.030	.003	.008	.014	.015
1923		.003	.007	.015	.017
Average	.030	.003	.008	.015	.016
	Percentage of total nitrogen of oven-dried rice				
Wataribune:					
1925	1.22	0.14	0.21	0.72	0.82
1923		.14	.27	.77	.87
Average	1.22	.14	.24	.75	.85
Blue Rose:					
1925	1.91	.16	.23	1.38	1.64
1923		.17	.22	1.42	1.57
Average	1.91	.17	.23	1.40	1.61
Honduras:					
1925	2.15	.24	.56	.98	1.07
1923		.21	.52	1.06	1.23
Average	2.15	.23	.54	1.02	1.15
	Percentage of water-soluble nitrogen of oven-dried rice				
Wataribune:					
1925	50.53	5.78	8.92	29.90	33.83
1923		5.98	11.28	31.96	36.25
Average	50.53	5.88	10.10	30.93	35.04
Blue Rose:					
1925	52.38	4.31	6.32	37.84	45.01
1923		4.54	6.01	38.93	43.20
Average	52.38	4.43	6.17	38.39	44.11
Honduras:					
1925	52.25	5.76	13.53	23.90	26.04
1923		5.19	12.58	25.88	29.84
Average	52.25	5.48	13.06	24.89	27.94

In Table 4 it will be noted that the differences in the nitrogen in the phosphotungstic precipitate of the several varieties are comparatively small. When calculated on the basis of the oven-dried kernel and on the basis of the total nitrogen of the oven-dried kernel, Wataribune and Honduras have, respectively, the lowest and highest percentage of nitrogen, while that in Blue Rose is between the two. The same relationship holds good for the nitrogen of acid amides, when calculated on the basis of the total nitrogen and of the water-soluble nitrogen. With regard to the percentage of humin nitrogen, it will be seen that it is highest in Honduras and lowest in Blue Rose, while that in Wataribune is between the two. The percentage of amino acid nitrogen is highest in Blue Rose (0.021 and 1.40); next in order come the percentages in Honduras (0.015 and 1.02) and Wataribune (0.010 and 0.75), calculated, respectively, on the oven-dried kernel and on the total nitrogen of the oven-dried kernel. The percentage of peptide nitrogen is highest in Blue Rose (0.025 and 1.61), next highest in Honduras (0.016 and 1.15), and lowest in Wataribune (0.011 and 0.85), calculated to the oven-dried kernel and to the total nitrogen of the oven-dried kernel.

GENERAL DISCUSSION AND INTERPRETATION OF RESULTS

TOTAL NITROGEN IN THE VARIOUS CEREALS

Table 5 needs some explanation. In order readily to compare the total nitrogen in different varieties, it was deemed best to consider the amount of nitrogen in the variety of each cereal richest in nitrogen as 100. It is to this basis that the amounts of nitrogen in the other varieties were calculated. Further, in order to have a broad general basis for comparing the total nitrogen in all of the cereals studied, the highest percentage of nitrogen, which was found to occur in Marquis (3.04 per cent), calculated on the oven-dried wheat kernel, was considered (in column 6) as 100, and the nitrogen of the other cereals was compared with it.

TABLE 5.—*Proportion of total nitrogen in the ungerminated kernel of various cereals*

Sample No.	Kind and variety	Where and when grown	Total nitrogen calculated on the basis of—		
			Oven-dried cereal	Variety richest in nitrogen within each cereal considered as 100	Cereal richest in nitrogen considered as 100
	Wheat		*Per cent*	*Per cent*	*Per cent*
1	Fultz, C. I. 3598	Arlington Farm, Rosslyn, Va., 1920	1.80	59.21	59.21
2	Kanred, C. I. 5146	Hays Branch Experiment Station, Hays, Kans., 1922.	2.83	93.09	93.09
3	Kubanka, C. I.	1920 [a]	3.03	99.67	99.67
4	Marquis, C. I. 3641	Dickinson Substation, Dickinson, N. Dak., 1920.	3.04	100.00	100.00
	Oats				
5	Swedish Select, C. I. 134	Dickinson, N. Dak., 1920	2.59	100.00	85.20
6	Iowar, C. I. 847	Iowa Agriculture Experiment Station, Ames, Iowa, 1922.	2.40	92.66	78.95
7	Victory, C. I. 560	Dickinson, N. Dak., 1922	2.02	77.99	66.45
8	Winter Turf, C. I. 435-4	Arlington Farm, Rosslyn, Va., 1922	1.50	57.91	49.34
	Corn				
9	Four County	Iowa Agriculture Experiment Station, Ames, Iowa, 1921.	1.70	100.00	55.92
10	United States Selection No. 77.	Piketon, Ohio, 1919	1.59	93.53	52.30
11	Hall Gold Nugget Selection No. 193.	Rhineback, N. Y., 1921	1.46	85.88	48.03
	Rye				
12	North Dakota No. 959	Dickinson, N. Dak., 1922	2.41	100.00	79.28
13	Von Rümker, C. I. 133	1923 [a]	1.84	76.35	60.53
14	Reg. Rosen No. R 22198	Parma, Mich., 1922	1.65	68.46	54.28
	Rice				
15	Wataribune, C. I. 1561	Sacramento Valley, Calif., 1923	1.18	69.41	38.82
16	Blue Rose, C. I. 1962	Stuttgart, Ark., 1923	1.30	76.47	42.76
17	Blue Rose, C. I. 1962	Rice Experiment Station, Crowley, La., 1923.	1.39	81.76	45.72
18	Honduras, C. I. 1643	do	1.70	100.00	55.92
19	Fortuna, C. I. 1344	do	1.53	90.00	50.33
20	Wataribune, C. I. 1561	Rice Experiment Station, Crowley, La., 1925.	1.24	72.94	40.79
21	Blue Rose, C. I. 1962	do	1.51	88.82	49.67
22	Honduras, C. I. 1643	do	1.38	81.18	45.39
23	Fortuna, C. I. 1344	do	1.46	85.88	48.03

[a] The place where grown could not be ascertained.

Inspection of column 5 of Table 5 shows marked differences in the total nitrogen of the several varieties of each cereal. Thus, of the corn varieties studied, Four County corn has the highest nitrogen content (100 per cent), and United States Selection No. 77 (93.53 per cent) and Hall Gold Nugget Selection No. 193 (85.88 per cent) rank next in the order named. On the other hand, when the various cereals are compared (column 6) it will be found that there are characteristic differences among them. Thus, the wheat and rice kernels have, respectively, the highest and lowest nitrogen content, and between the two lie the percentages of nitrogen in oat, rye, and corn kernels, which decrease in the order named. These differences in the nitrogen content of the various cereals may be due, aside from inheritance, to a variety of causes, such as water and starch content of the kernels, quality of the seed, soil and climatic conditions, and last but not least, the method used for the nitrogen determination. It is evident that, other considerations being equal, the kernels should be the poorer in nitrogen the more water and starch they contain, because in this case they may be spoken of as kernels with a "dilute" content as far as the protein nitrogen is concerned. On the other hand, the kernels should be richer in nitrogen the more nitrogen there is available in the soil producing them, the higher the quality of the seed, and the more favorable the climatic conditions. Inasmuch as the total nitrogen in all the cereals investigated was calculated on the basis of the oven-dried kernel and the method employed for the nitrogen estimation was practically identical throughout these investigations, the water content and method do not play here any special rôle. The starch content will not be considered here because its determination was not contemplated in our work, nor will the quality of the various seeds as to which no information was obtained be taken into consideration. As to the powerful influences of soil and climate, they can best be illustrated by a comparison of the Blue Rose rices grown at Stuttgart, Ark., in 1923 (No. 16) and at Crowley, La., in 1923 (No. 17), with that grown at Crowley, La., in 1925 (No. 21). They had, respectively, 76.47, 81.76, and 88.82 per cent of nitrogen, considering the quantity of nitrogen in 1923 as 100. Likewise, Honduras (No. 18) and Fortuna (No. 19) from Crowley, La., the nitrogen in which in 1923 was assigned the comparative values of 100 and 90 per cent, respectively, had in 1925, on the same basis, but 81.18 (No. 22) and 85.88 (No. 23) per cent, respectively.

PROTEIN NITROGEN AND NONPROTEIN NITROGEN IN THE VARIOUS CEREALS

Table 6 shows that protein nitrogen makes up the great bulk of the total nitrogen, and ranges, on an average, from about 87, 88, and 90 per cent in rye, wheat, and oats, respectively, to more than 95 per cent in corn and rice. While the proportion of protein nitrogen is smaller in rye, wheat, and oats than in corn and rice, the absolute quantity of protein nitrogen, and hence of proteins, is considerably larger in the first three cereals mentioned because of their higher percentage of total nitrogen than it is in the latter two cereals. Inasmuch as the total nitrogen in the kernel of the various cereals is, aside from inheritance, attributable more or less to factors such as soil, climate, and quality of seed, it seems not unreasonable to ascribe to the same causes also the greater or smaller proportion of protein nitrogen in the various kernels.

TABLE 6.—*Proportion of protein and of nonprotein nitrogen in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Protein nitrogen, calculated on the basis of—		Nonprotein nitrogen, calculated on the basis of—	
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Oven-dried kernel	Total nitrogen of oven-dried kernel
	Wheat		*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Fultz, C. I. 3598	Arlington Farm, Rosslyn, Va., 1920	1.64	91.76	0.15	8.24
2	Kanred, C. I. 5146	Hays Branch Experiment Station, Hays, Kans., 1922	2.47	87.01	.37	12.99
3	Kubanka, C. I.	1920 *	2.73	86.73	.40	13.27
4	Marquis, C. I. 3641	Dickinson Substation, Dickinson, N. Dak., 1920	2.65	87.26	.39	12.74
	Oats					
5	Swedish Select, C. I. 134	Dickinson, N. Dak., 1920	2.30	88.65	.29	11.35
6	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922	2.17	90.52	.23	9.48
7	Victory, C. I. 560	Dickinson, N. Dak., 1922	1.82	90.21	.20	9.79
8	Winter Turf, C. I. 435-4	Arlington Farm, Rosslyn, Va., 1922	1.36	91.03	.14	8.97
	Corn					
9	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921	1.63	95.70	.073	4.30
10	United States Selection No. 77	Piketon, Ohio, 1919	1.52	95.69	.068	4.31
11	Hall Gold Nugget Selection No. 193	Rhinebeck, N. Y., 1921	1.39	95.59	.064	4.41
	Rye					
12	North Dakota No. 959	Dickinson, N. Dak., 1922	2.02	83.89	.39	16.11
13	Von Rümker, C. I. 133	1923 *	1.58	85.73	.26	14.27
14	Reg. Rosen, No. R 22198	Parma, Mich., 1922	1.51	91.52	.14	8.48
	Rice					
15	Wataribune, C. I. 1561	Rice Experiment Station, Crowley, La., 1925	1.18	95.33	.062	5.02
16	Blue Rose, C. I. 1962	do	1.45	95.92	.060	4.00
17	Honduras, C. I. 1643	do	1.34	97.12	.055	4.02
18	Fortuna, C. I. 1344	do	1.37	94.17	.094	6.46

* The place where grown could not be ascertained.

There is, however, one factor which may have considerably more influence upon the proportion of protein nitrogen in the cereal kernel than the conditions mentioned above, namely, the greater or lesser ripeness of the seed. From observations of Schulze (*19*, *20*) and other investigators the conclusion seems to be justified that the proportion of proteins and nonproteins is more or less fluctuating, depending on the state of ripeness of the seed. Unripe seed ordinarily contains a higher percentage of nonproteins and ripe seed ordinarily contains a higher percentage of proteins, and vice versa. Unfortunately, there is no information as to the state of ripeness at the time of harvest of any of the varieties investigated.

That the nonprotein nitrogen, which was either directly estimated in the filtrate from the protein nitrogen according to Stutzer's method (*22*), or calculated by difference from 100, would stand in reverse ratio to the protein nitrogen, was to be expected.

ACID AMIDE NITROGEN AND HUMIN NITROGEN IN THE VARIOUS CEREALS

In Table 7 it is seen that in general rye and rice have the highest and lowest percentages of acid amide nitrogen, respectively, the intermediate cereals, wheat, corn, and oats, showing, in the order named, a gradual decrease in percentage of acid amide nitrogen.

Yet the absolute quantity of the nitrogen corresponding to acid amides is very small. As will be remembered, the total nonprotein nitrogen in the various cereals (Table 6) ranges, in round figures, from about 4 or 5 per cent in corn and rice to about 10, 12, and 13 per cent in oats, wheat, and rye, respectively, calculated on the basis of the total nitrogen. Of this small percentage of nonprotein nitrogen, however, only part can be extracted with water, which part is distributed among the various nitrogenous compounds, such as acid amides, amino acids, and polypeptides. Nevertheless, their presence in the ungerminated kernel of the cereals seems to be physiologically important, as will subsequently be seen.

TABLE 7.—*Percentage of acid amide and humin nitrogen in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Acid amide nitrogen, calculated on the basis of—			Humin nitrogen, calculated on the basis of—			Remarks
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	
	Wheat		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	
1	Fultz, C. I. 3598	Arlington Farm, Rossyln, Va., 1920.	0.026	1.46	8.76				Cold-water extract.
2	Kanred, C. I. 5146.	Hays Branch Experiment Station, Hays, Kans., 1922.	.053	1.88	12.99				Do.
3	Kubanka, C. I.	1920[a]	.052	1.72	12.61				Do.
4	Marquis, C. I. 3641.	Dickinson substation, Dickinson, N. Dak., 1920.	.058	1.91	12.33				Do.
	Oats								
5	Swedish Select, C. I. 134.	Dickinson, N. Dak., 1920.	.051	1.95	13.69				Do.
6	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922.	.051	2.13	13.45				Hot-water extract.
			.046	1.93	12.18				Cold-water extract.
7	Victory, C. I. 560.	Dickinson, N. Dak., 1922.	.032	1.61	12.03				Hot-water extract.
			.029	1.45	10.86				Cold-water extract.
8	Winter turf, C. I. 435–4.	Arlington Farm, Rosslyn, Va., 1922.	.027	1.80	15.01				Do.
	Corn								
9	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	.034	2.00	16.58				Hot-water extract.
10	United States Selection No. 77.	Piketon, Ohio, 1919	.018	1.13	11.64				Cold-water extract.
11	Hall Gold Nugget Selection No. 193.	Rhinebeck, N. Y., 1921.	.021	1.44	11.15				Hot-water extract.
			.022	1.51	11.32				Cold-water extract.
	Rye								
12	North Dakota No. 959.	Dickinson, N. Dak., 1922.	.090	3.72	16.32				Hot-water extract.
13	Von Rümker, C. I. 133.	1923[a]	.093	4.96	18.50				Do.
14	Reg. Rosen, No. R. 22198.	Parma, Mich., 1922	.069	4.14	14.28				Do.
	Rice								
15	Wataribune, C. I. 1561.	Rice Experiment Station, Crowley, La., 1925.	.002	.14	5.88	0.003	0.24	10.10	Do.
16	Blue Rose, C. I. 1962.	do	.003	.17	4.43	.003	.23	6.17	Do.
17	Honduras, C. I. 1643.	do	.003	.23	5.48	.008	.54	13.06	Do.

[a] The place where grown could not be ascertained.

Table 7 shows that Honduras and Blue Rose have the highest and lowest proportion of humin nitrogen, respectively, the proportion in Wataribune being between the two. The absolute quantity of humin nitrogen, too, is very small, as has already been pointed out. The formation of humin bodies is chiefly, if not wholly, due to acid hydrolysis incidental to the estimation of acid amides (and polypeptides), and is to be ascribed to partial decomposition of tryptophan, tyrosine, cystine, and diamino acids present in plant and other biological materials, as was pointed out by Gortner and Holm (*5*) as well as by Roxas (*18*).

AMINO NITROGEN AND PEPTIDE NITROGEN IN THE VARIOUS CEREALS

By referring to Table 8 it will be noted that rice and rye have, respectively, the lowest and highest proportions of amino nitrogen, and that between them are wheat, oats, and corn, in which, in the order named, the proportion of amino nitrogen, calculated on the basis of the oven-dried kernel and of the total nitrogen of the oven-dried kernel, gradually increases. It will also be found that rice and rye have the lowest and highest percentage of peptide nitrogen, respectively, but that between the two are corn, oats, and wheat, in which, in the order named, the peptide nitrogen, calculated to the oven-dried kernel and to the total nitrogen of the oven-dried kernel, increases.

While the absolute quantity of amino and of peptide nitrogen is very small for the reasons outlined in connection with the acid amide and humin nitrogen, they all appear to be of considerable physiological importance. During the first two or three days of seed germination some soluble, diffusible, and readily transportable material is needed for building up the tissues of the young plantlet. The nonproteins contained in the seed represent such material. As soon, however, as chlorophyll appears and the enzymes are coming into play the plantlet is, of course, able to synthesize carbohydrates and to split the stored-up seed proteins, which are mostly insoluble and at any rate not diffusible, into smaller molecules (amino acids, acid amides, etc.), which can then be readily transported to the growing parts of the seedlings. The polypeptides, standing as they do between the amino acids on the one hand and the proteins on the other, may be split by the enzymes present into amino acids for translocation to points where they are needed, or they may represent the immediate material out of which the great protein structures are synthesized. From the standpoint of nutrition, the amino acids play an important rôle, since it has been shown by recent investigations of Osborne and Mendel (*13*) and Abderhalden (*1*) that the animal organism can build up proteins out of amino acids.

TABLE 8.—*Percentage of amino and peptide nitrogen in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Amino nitrogen, calculated on the basis of—			Peptide nitrogen, calculated on the basis of—		
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel
	Wheat		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	Fultz, C. I. 3598	Arlington Farm, Rosslyn, Va., 1920.	0.033	1.85	11.13	0.084	4.67	[a] 28.09
			.029	1.61	9.72			
			.033	1.85	11.12			
2	Kanred, C. I. 5146	Hays Branch Experiment Station, Hays, Kans, 1922.	.066	2.32	16.04	.111	3.89	[a] 26.86
			.067	2.38	16.46			
3	Kubanka, C. I.	1920[c]	.047	1.55	11.41	.155	3.13	[a] 37.76
			.037	1.21	8.92			
			.039	1.28	9.41			
4	Marquis, C. I. 3641	Dickinson Substation, Dickinson, N. Dak., 1920.	.048	1.56	10.09	.151	4.98	[a] 32.20
			.060	1.95	12.63			
			.055	1.81	11.67			
	Oats							
5	Swedish Select, C. I. 134.	Dickinson, N. Dak., 1920	.064	2.48	17.39	.106	4.10	[a] 28.71
6	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922.	.057	2.35	14.87	.098	4.07	[b] 25.69
						.084	3.49	[a] 22.03
7	Victory, C. I. 560	Dickinson, N. Dak., 1922	.040	2.02	15.08	.077	3.82	[b] 28.58
						.073	3.63	[a] 27.16
8	Winter Turf, C. I. 435-4.	Arlington Farm, Rosslyn, Va., 1922.	.025	1.65	13.79	.032	2.15	[a] 17.88
	Corn							
9	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	.044	2.59	21.68	.070	4.12	[b] 34.36
10	United States Selection No. 77.	Piketon, Ohio, 1919	.042	2.64	28.11	.052	3.27	[a] 34.11
11	Hall Gold Nugget Selection No. 193.	Rhinebeck, N. Y., 1921	.052	3.56	27.57	.035	2.40	[b] 18.55
			.050	3.43	26.51	.039	2.67	[a] 20.66
	Rye							
12	North Dakota No. 959.	Dickinson, N. Dak., 1922	.075	3.09	13.56	.162	6.69	[b] 29.36
13	Von Rümker, C. I. 133.	1923[a]	.101	5.39	20.10	.155	8.30	[b] 30.96
14	Reg. Rosen No. R, 22198.	Parma, Mich., 1922	.099	5.97	20.62	.070	4.21	[b] 14.55
	Rice							
15	Wataribune, C. I. 1561.	Rice Experiment Station, Crowley, La., 1925.	.010	.75	30.93	.011	.85	[b] 35.04
16	Blue Rose, C. I. 1962	do	.021	1.40	38.39	.025	1.61	[b] 44.11
17	Honduras, C. I. 1643	do	.015	1.02	24.89	.016	1.15	[b] 27.94

[a] Cold-water extract. [b] Hot-water extract. [c] The place where grown could not be ascertained.

BASIC NITROGEN AND THE ASH IN THE VARIOUS CEREALS

In Table 9, with "basic nitrogen" is included all the nitrogen obtained in the phosphotungstic precipitate, which may contain not only ammonia and the hexon bases (histidine, arginine, lysine), but also proteins, proteoses, and peptones. Table 9 shows that oats and rice have, respectively, the highest and lowest proportion of basic nitrogen, calculated on the basis of the oven-dried kernel and of the total nitrogen of the oven-dried kernel, the proportion in corn being between the two. It is worthy of note that the basic nitrogen makes up a considerable proportion of the water-soluble nitrogen, being greater than the percentage of any of the nonprotein nitrogenous

compounds, such as amino acids, acid amides, etc. With regard to the ash, it will be seen that oats has the highest percentage of ash, followed by the percentages in rye, corn, and rice in the order named.

TABLE 9.—*Percentage of basic nitrogen and of ash in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Basic nitrogen, calculated on the basis of—			Ash, calculated on the basis of—	
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	Air-dried kernel	Oven-dried kernel
	Oats		*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	Swedish select, C. I. 134	Dickinson, N. Dak., 1920	0.165	6.40			3.25
2	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922.					3.39
3	Victory, C. I. 560	Dickinson, N. Dak., 1922	.111	5.48			3.15
4	Winter Turf, C. I. 435-4	Arlington Farm, Rossyln, Va., 1922.	.078	5.21			3.72
	Corn						
5	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	.076	4.47	37.35		1.40
6	United States Selection No. 77.	Piketon, Ohio, 1919	.061	3.84	40.11		1.33
7	Hall Gold Nugget Selection No. 193.	Rhinebeck, N. Y., 1921	.076	5.21	39.57		1.33
	Rye						
8	North Dakota, No. 959	Dickinson, N. Dak., 1922					1.79
9	Von Rümker, C. I. 133	1923 [a]					2.11
10	Reg. Rosen, No. R 22198	Parma, Mich., 1922					2.04
	Rice						
11	Wataribune, C. I. 1561	Sacramento Valley, Calif., 1923.				1.30	1.39
12	Blue Rose, C. I. 1962	Stuttgart, Ark., 1923				1.25	1.36
13	do	Rice Experiment Station, Crowley, La., 1923.				1.20	1.31
14	Honduras, C. I. 1643	do				1.23	1.36
15	Fortuna, C. I. 1344	do				0.89	0.97
16	Wataribune, C. I. 1561	Rice Experiment Station, Crowley, La., 1925.	.015	1.22	50.53	.86	.93
17	Blue Rose, C. I. 1962	do	.029	1.91	52.38	1.11	1.19
18	Honduras, C. I. 1643	do	.030	2.15	52.25	1.44	1.54
19	Fortuna, C. I. 1344	do				1.01	1.09

[a] The place where grown could not be ascertained.

Owing to their great complexity, the quantitative separation of the various compounds in cereals involves a number of difficulties. For this reason the data here presented can not be considered as perfectly quantitative. Yet when due allowance is made for unavoidable errors in such work, it is nevertheless true that if all the conditions and manipulations have been strictly identical throughout the investigation, as was the endeavor in this instance, the results are decidedly comparable.

SUMMARY

Of the rice varieties of the crop of 1923 studied, Honduras was characterized by the highest percentage of total nitrogen, 1.70. Fortuna had the next highest, 1.53, while the other varieties had

smaller percentages, Blue Rose from Louisiana, 1.39, Blue Rose from Arkansas, 1.30, and Wataribune, 1.18. Of the varieties of the 1925 crop studied, Blue Rose from Louisiana had the highest percentage of nitrogen, 1.51, Fortuna having 1.46, Honduras 1.38, and Wataribune 1.24.

It has been shown in this paper that polypeptides and free amino acids occur in the ungerminated rice kernel (brown rice).

The proportions of amino nitrogen in the varieties Wataribune, Blue Rose, and Honduras (all from Louisiana, crop of 1925) were, respectively, 0.010, 0.021, and 0.015 per cent, calculated on the oven-dried kernel, and 0.75, 1.40, and 1.02 per cent, calculated on the basis of the total nitrogen of the oven-dried kernel.

The acid amide nitrogen in the varieties Wataribune, Blue Rose, and Honduras (crop of 1925) constituted, respectively, 0.14, 0.17, and 0.23 per cent of the total nitrogen of the oven-dried kernel.

The nitrogen of polypeptides in the varieties Wataribune, Blue Rose, and Honduras (crop of 1925) made up, respectively, 0.011, 0.025, and 0.016 per cent of the oven-dried kernel, and 0.85, 1.61, and 1.15 per cent of the total nitrogen of the oven-dried kernel.

Considering the percentage of total nitrogen in Honduras (1.70), crop of 1923, as 100, it was found that Wataribune from California, Blue Rose from Arkansas, Blue Rose from Louisiana, and Fortuna—all of the 1923 crop—had, respectively, 69.41, 76.47, 81.76, and 90.00 per cent of nitrogen, while for the 1925 crop Wataribune from Louisiana and Honduras, Fortuna, and Blue Rose from Louisiana had, respectively, 72.94, 81.18, 85.88, and 88.82 per cent of nitrogen.

A comparison of the nitrogen compounds occurring in rice with those in wheat, rye, oats, and corn is given and their significance pointed out.

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PERIDERMIUM KURILENSE DIET. ON PINUS PUMILA PALL., AND PERIDERMIUM INDICUM N. SP. ON PINUS EXCELSA WALL.[1]

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INTRODUCTION

There appear to be only three species of Peridermium which attack the stems of five-needled or white pines: *Cronartium ribicola* Fischer (*Peridermium strobi* Kleb.) on *Pinus albicaulis* Engelmann, *P. aristata* Engelmann, *P. ayacahuite* Ehrenb., *P. balfouriana* Balfour, *P. cembra* L., *P. excelsa* Wall., *P. flexilis* James, *P. koraiensis* Sieb and Zucc., *P. lambertiana* Douglas, *P. monticola* Douglas, *P. parviflora* Sieb and Zucc., *P. peuce* Gris., *P. strobiformis* Engelmann, and *P. strobus* L. (*5*, *6*)[2][3]; *Peridermium kurilense* Diet. (*4*) on *Pinus pumila* Pall.; and *Peridermium indicum* n. sp. on *Pinus excelsa*. The life history, morphology, and parasitism of *Cronartium ribicola* are now fairly well known. Either or both of the other two species, *Peridermium kurilense* and *P. indicum*, might become serious parasites if introduced into this country. At any rate their invasion would certainly complicate the problem of differential diagnosis in the western part of the United States, where *Cronartium ribicola* and *C. occidentale*, the piñon pine rust, are already present.

SOURCE OF MATERIAL

The material of *Peridermium kurilense* available for study consisted of one small piece of bark measuring approximately 17×10×3 mm., bearing a few broken aecia. The specimen is No. 19047 in the herbarium of J. R. Weir. It is labelled as type material.

Two specimens of the species which is here named *Peridermium indicum* were lent for examination through the courtesy of E. J. Butler and W. McRae. The first specimen was sent by Butler to W. Stuart Moir, in 1921, and subsequently the writers had an opportunity to see it. The second specimen, sent directly from Pusa, India, by McRae, consisted of a single piece of twig about 2½ inches long and one-quarter of an inch in diameter, bearing 14 aecia. Both specimens were part of the same collection. The label with the second specimen reads, "*Peridermium complanatum* Barc. on *Pinus excelsa*. Kulu, N. W. Himalaya. 3–6–14 (June 3, 1914). Coll. R. S. Troup." However, Barclay's original description (*1*) of *Aecidium complanatum* refers definitely to an aecium on the needles of *Pinus longifolia* Roxb.; and the name is therefore not applicable to the stem *Peridermium* on *Pinus excelsa*.

[1] Received for publication Nov. 17, 1926; issued April, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 330.

[3] SPAULDING, P. NOTES UPON THE WHITE PINE BLISTER RUST IN EUROPE AND UPON CONDITIONS AFFECTING ITS STATUS THERE. [Unpublished manuscript.]

METHODS AND MEASUREMENTS

The aeciospores were mounted for study in the glycerin and glycerin-jelly mounting media used for mounting the urediniospores and aeciospores of *Cronartium ribicola* and *C. occidentale* (*2*, *3*). All measurements were made with a filar micrometer. The thickness of wall reported refers to the thickest part of the side wall of the spores. The peridial cells were studied in sectional view. The wall thickness of the peridial cells refers to the wall thickness of the

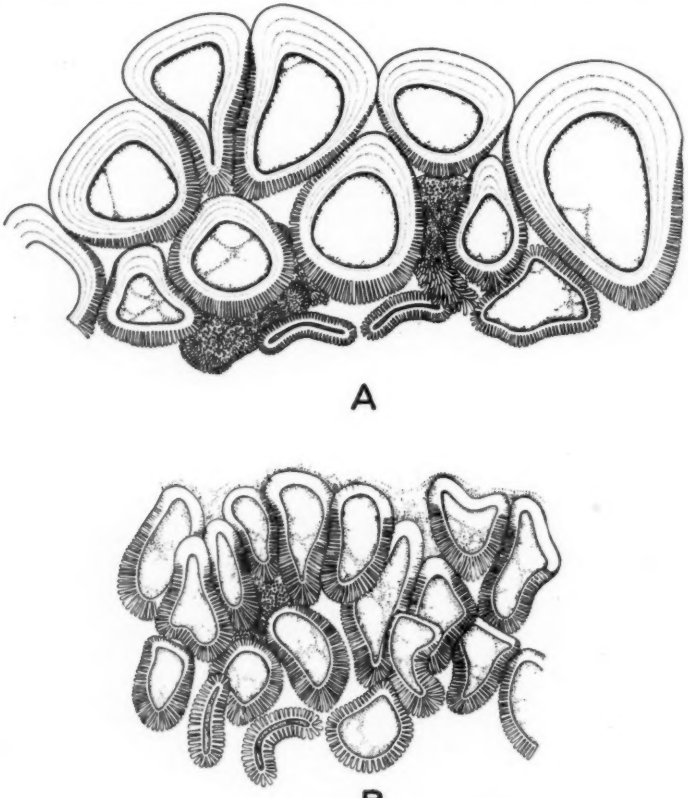

FIG. 1.—Sections through the peridia in the region near the dome: A, *Peridermium kurilense*; B, *Peridermium indicum*. × 625

outer or outside wall of the outer layer of cells in the peridium. The results of the measurement study are presented in Table 1. For the sake of comparison, data on species means of *C. ribicola* and *C. occidentale* (*3*) are also included.

Sectional views of the peridial cells made from material which appeared to be normal for the specimens are shown in Figure 1, A and B. The drawings do more to bring out the differences between the two species than any amount of description; and a comparison of the illustrations with others representing similar sections

from the peridia of *Cronartium ribicola* and *C. occidentale* (*3*) yields convincing evidence of the value of peridial-cell characters in diagnosis.

TABLE 1.—*Data on the size of the aeciospores, aeciospore tubercles, and peridial cells of Peridermium kurilense, P. indicum, Cronartium ribicola, and C. occidentale*

Aeciospores, aeciospore tubercles, and peridial cells	Basis	Length		Width		Wall		Ratio
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean length / Mean width
Aeciospores:		μ		μ		μ		
Peridermium kurilense	200	27.3	3.5	21.4	2.3	4.24	0.89	1.27
Peridermium indicum	200	27.0	3.4	20.3	2.3	4.33	.93	1.33
Cronartium ribicola	(a)	24.2	2.26	18.3	1.66	3.41		1.32
Cronartium occidentale	(a)	26.8	2.79	19.0	2.12	3.85		1.41
Aeciospore tubercles, end view:								
Peridermium kurilense	100	1.43	.31	1.05	.23			1.36
Peridermium indicum	100	1.41	.28	1.05	.23			1.34
Cronartium ribicola	(a)	1.52	.31	1.10	.21			1.38
Cronartium occidentale	(a)	2.29	.64	1.33	.27			1.72
Peridial cells:								
Peridermium kurilense	200	53.3	8.0	38.3	7.7	13.77	2.98	1.39
Peridermium indicum	200	38.4	5.9	24.9	4.6	3.69	.91	1.54
Cronartium ribicola	(a)	41.4	5.5	28.5	5.3	7.16	1.36	1.45
Cronartium occidentale	(a)	27.4	4.1	19.1	3.5	4.68	.89	1.43

[a] Species means based on numerous specimens. For detailed data see (*3*).

DESCRIPTION

The following descriptions combine the biometric and morphologic data:

Peridermium kurilense Diet.

Aecia usually discrete, occasionally confluent.

Peridia apparently persistent, 2 to 3, occasionally 4, cells thick; outer layer of cells in region near dome, sectional view, long and short dimensions (basis 200 measurements from 1 specimen) 53.3×38.3μ, standard range [4] 45.4−61.3×30.6−46.0μ, ratio mean length divided by mean width 1.39; outer wall of cells in outer layer smooth, 13.77μ thick, generally somewhat thicker than inner wall of the same cells; walls striated; inner wall marked with thin tubercles which are 5 to 10 times as long as they are broad; walls of cells in the second layer marked with tubercles over their entire surface, the tubercles, however, being very short on the ends of the cells toward the outside of the peridium and full length on the ends of the cells toward the inside of the peridium; outer walls of the cells in the second layer often thicker than the inner walls of the same cell; walls of cells in third layer generally uniform in thickness, marked over entire surface with uniformly distributed tubercles.

Aeciospores obovoid to ellipsoid, sometimes subspherical, generally smoothly curved in outline, (basis 200 spores) 27.3×21.4μ; standard range 23.8−30.8×19.1−23.7μ; ratio mean length divided by mean width 1.27; wall 4.24μ thick, standard range 3.35−5.13μ; wall partly smooth and partly marked with tubercles, the smooth area fissured near junction with tubercles; tubercles fairly regular in outline in view, (basis 100 measurements) 1.43×1.05μ, standard range 1.12−1.47×0.82−1.28μ; ratio mean length divided by mean width 1.36.

On twigs of *Pinus pumila* Pall. (=*P. cembra* var. *pumila* Pall.), Kurile Island, Japan. Specimen No. 19047 Herb. J. R. Weir.

Peridermium indicum n. sp.

Aecia usually discrete.

Peridia persistent, 3 to 4 cells thick; outer layer of cell in region near dome, sectional view, long and short dimensions (basis 200 measurements) 38.4×24.9μ,

[4] The lower and upper limits of the standard range differ from the mean by the amount of the standard deviation.

standard range 32.5−44.3×20.3−29.5μ, ratio mean length divided by mean width 1.54; outer wall of cells in outer layer smooth, 3.69μ thick, generally somewhat thinner than the inner wall plus its tubercles; outer wall sometimes fissured but bearing no tubercles; inner wall marked with thin tubercles which are five to ten times as long as they are broad; walls of the second and third layers of cells marked essentially the same as the cells in the outer layer, except that the walls on the outer ends of the cells are sometimes studded with short tubercles; walls of cells in the inner layer nearly uniform in thickness, marked with uniformly distributed tubercles.

Aeciospores obovoid to ellipsoid, generally smoothly curved in outline, (basis 200 spores) 27.0×20.3μ, standard range 23.6−30.4×18.0−22.6μ; ratio mean length divided by mean width 1.33; wall 4.33μ thick, standard range 3.40−5.26μ; wall partly smooth and partly marked with tubercles, the smooth area fissured near junction with tubercles; tubercles fairly regular in outline in end view, (basis 100 measurements) 1.41×1.05μ, standard range 1.13−1.69×0.82−1.28μ; ratio mean length divided by mean width 1.34.

On twigs and branches of *Pinus excelsa* Wall., Kulu, N. W. Himalaya, India, collected by R. S. Troup, June 3, 1914. Specimen from the Pusa herbarium.

Through the courtesy of E. J. Butler one-half of the smaller specimen of *Peridermium indicum* has been deposited in the Pathological Collections of the Bureau of Plant Industry, United States Department of Agriculture.

POINTS OF SIMILARITY AND MEANS OF DIFFERENTIATION

Dietel (4) has already noted the similarity between the habit of *Peridermium kurilense* and the aecial stage of *Cronartium ribicola*. This resemblance is also to be observed between *P. indicum* and *C. ribicola*. The aeciospores of the two Asiatic specimens examined are very similar. Their relatively large size is obvious. Their position with respect to the specimen means of *C. ribicola* and *C. occidentale* may be determined easily by plotting their mean spore sizes on Figure 2 in the earlier paper (3). Both appear to be well without the *C. ribicola* range; but they might be confused with *C. occidentale*. The characters of the peridial cells are so different, however, that separation of the four species on the basis of these characters alone seems to be a relatively simple and certain method of diagnosis.

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NEW SPECIES OF CHALCID FLIES PARASITIC ON THE GIPSY-MOTH PARASITE, APANTELES MELANOSCELUS (RATZEBURG)[1]

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INTRODUCTION

In the course of investigations by the writer and S. M. Dohanian, of the gipsy moth and brown-tail moth project, the new species of chalcid flies described in this paper were found to be parasitic on the gipsy-moth parasite, *Apanteles melanoscelus* (Ratzeburg).

During the seasons of 1922, 1923, and 1924 an exhaustive study of the hyperparasites of *Apanteles melanoscelus* was made, not only to learn their biology and interrelationships, but also to determine the exact extent to which this useful parasite of the notorious gipsy moth, *Porthetria dispar* L., is subject to attack by secondary parasites. No less than 35 such enemies were discovered during the period of the investigations, only a few of which, however, were found to be of importance in reducing the activities of *melanoscelus*. *A. melanoscelus* is one of the most important of the several parasites of the gipsy moth which the Bureau of Entomology has successfully introduced and established in the gipsy-moth infested area of the New England States. It has two generations annually, both on the larvae of its host. Despite a long array of enemies, it has spread widely over the infested area and is aiding efficiently in reducing the ravages of the gipsy moth.

FAMILY PTEROMALIDAE

Coelopisthia scutellata, n. sp.

Female.—Length, 2 millimeters. Head transverse, much broader than thorax, and viewed from in front rather rounded, a little broader than long; eyes large, elongate-oval, bare, diverging a little below; entire head closely reticulately punctate, the face below antennae strongly receding and with very small but deeply impressed and closely set punctures; above the insertion of the antennae the punctures become gradually larger and more shallow toward vertex; clypeus more shining than lower part of face, its anterior margin weakly sinuate; scape of antenna slender, cylindrical, extending nearly to the median ocellus; pedicel long and slender, broadening apically, and distinctly longer than the two ring-joints and the first segment of funicle combined; first ring-joint very short, strongly tranverse; the second more than twice as long as the first, but still distinctly broader than long; funicle broadening very slightly apically; first funicle segment as long as broad, the following subequal and slightly broader than long; club acuminate, with the two basal segments about as long as the apical segments of funicle, the last segment shorter; lateral ocelli very slightly nearer to the median ocellus than to the eye margin; postocellar line at least one and one-half times the ocell-ocular line; pronotum closely punctate, a little more coarsely so than the head; mesoscutum very evenly punctate and sub-opaque, the punctures deeper and smaller than on the head, smaller anteriorly than behind; axillae and scutellum closely covered with minute, deep, almost

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confluent punctures, and opaque; the much finer punctation of the axillae and scutellum contrasts strongly with that of the mesoscutum; propodeum strongly punctate, the lateral folds complete, the median carina wanting; propodeal spiracles oval; neck of propodeum very short and provided with a conspicuous margined fovea at either side; pleura closely punctate and subopaque; anterior wings with postmarginal vein slightly shorter than stigmal vein, the latter distinctly less than half as long as the marginal vein; marginal cilia wanting; basal third of wing hairless, the remainder with very short hairs; abdomen ovate, pointed at tip, smooth, and polished. Head and thorax mostly bluish black; antennal scape yellow, remainder of antennae black; posterior margin of pronotum somewhat aeneous; scutellum, and the axillae to a smaller degree, bronzy green, contrasting strikingly with the color of mesoscutum; propodeum blue; anterior wings with a large deeply fuscous patch extending across wing from the base of marginal vein to a point beyond the end of stigmal vein; anterior coxae green or blue on the outer face; the middle pair mostly brownish, greenish only at base; the posterior pair blue; remainder of legs mostly brownish yellow; the posterior femora more or less dusky, especially along dorsal margin and at apex; the middle and hind tibiae brownish black, usually a little paler at base; and all tarsi pale yellow except for the dusky apical segment; abdomen dark aeneous, with slight purplish or greenish reflections at base.

Male.—In general like the female, but the face is bright green, the funicle of antenna is brown, and the fuscous spot in the anterior wing is much less pronounced.

Type.—Cat. No. 28123, U.S.N.M.

Type locality.—Franklin, N. H.

Host.—*Apanteles melanoscelus* (Ratzeburg).

Described from six females and six males reared under Gipsy Moth Laboratory No. 12599 C.

Hypopteromalus, inimicus n. sp.

Readily distinguished from *tabacum* (Fitch), with which it is frequently associated as a parasite of *Apanteles melanoscelus*, by the more slender antennal segments, the much darker coxae, the usually brighter green coloring of the thorax, and the longer and narrower abdomen of the female.

Female.—Length, 3 millimeters. Head strongly transverse, a little broader than the thorax, entirely deeply closely punctate; clypeus striate, its anterior margin deeply sinuate; eyes elongate-oval, moderate; antennae inserted a little above the level of the lower eye margin; scape slender, cylindrical, extending very nearly to the median ocellus; pedicel shorter than first segment of funicle; the two ring-joints very short, strongly transverse, the second only slightly longer than the first; funicle cylindrical; the first segment one and one-half times as long as broad; the following becoming gradually shorter, the sixth apparently very slightly broader than long; club a little wider than funicle, the segments subequal, broader than long; lateral ocelli nearer to the anterior ocellus than to the eye margin; postocellar line longer than ocell-ocular line but less than one and one-half times as long; pronotum strongly transverse, sharply margined anteriorly; mesoscutum, scutellum, and axillae closely punctate, the punctures on the scutellum and axillae being a little smaller than those on the middle of the mesoscutum; propodeum punctate medially, smooth outside the lateral folds, which are complete; median carina wanting; neck of propodeum large, conspicuous, transversely wrinkled; propodeal spiracles large, strongly elliptical; marginal vein slightly longer than postmarginal, the latter one and one-half times the length of stigmal vein; stigmal knob small; abdomen ovate, about as long as the thorax, pointed at apex, smooth and shining. Green; antennae brown, with scape and apex of pedicel yellow, the funicle and club yellowish beneath; tegulae yellow; wings hyaline; legs, except all coxae which are completely dark green, wholly yellow; posterior femora sometimes faintly brownish above toward base; abdomen dark green, dark cupreous medially.

Male.—Head and thorax more brilliant green than in the female; legs much paler yellow; antennae entirely yellow and more slender, with all the segments of funicle longer than broad, at least the first two, and sometimes the first four, segments twice as long as broad; abdomen with a median yellow spot covering apex of first segment and part of second.

Type.—Cat. No. 28122, U.S.N.M.

Type locality.—Boylston, Mass.

Host.—*Apanteles melanoscelus* (Ratzeburg).

Described from six females and five males, all reared from the above host; the type, allotype, and one paratype are from Boylston, Mass., and bear Gipsy Moth Laboratory No. 11239 a 2; four paratypes are from Pelham, N. H., three of these having been reared under Gipsy Moth Laboratory No. 11238 n and one under No. 11239 b; and the remaining three paratypes are from Melrose Highlands, Mass., two bearing Gipsy Moth Laboratory No. 10647 c and one Gipsy Moth Laboratory No. 10608.

FAMILY EULOPHIDAE

Dimmockia pallipes, n. sp.

Exceedingly similar to *secundus* Cwfd., from which it can be distinguished, however, by the conspicuous bronzy luster of the scutellum and head, and by the generally more delicate sculpturing of the scutellum.

Female.—Length, 1.7 millimeters. Head transverse, very slightly wider than thorax; the temples narrow; the cheeks rather broad; upper part of frons and the vertex mostly smooth and shining, with only a faint indication of sculpture; lower part of face weakly shallowly punctured, the punctures not especially distinct; malar space and cheeks very delicately reticulate, with a suggestion of vertical lineolation; eyes rather large, very broad, short-oval, bare; distance between the median and lateral ocelli subequal with ocell-ocular line; postocellar line at least one and one-half times the ocell-ocular line; antennae inserted about on a level with the lower eye margin; scape rather slender, subcylindrical, slightly thickened in the middle, and very nearly half as long as pedicel, funicle, and club united; pedicel a little longer than broad at apex, but distinctly shorter than first segment of funicle; the single ring-joint very short, strongly transverse; funicle 4-segmented, the first segment the longest, nearly one and one-half times as long as broad; the second, third, and fourth segments of about equal length, the third and fourth slightly broader than the second, usually subquadrate; club acuminate, 3-segmented, the segments poorly defined, the first and second transverse; pronotum conical, faintly punctured; mesoscutum shining, shallowly punctate, the punctures of the posterior part larger and deeper than on the anterior part; scutellum and axillae much more finely punctured, the punctures very small and elongate, and so arranged that the axillae and the scutellum laterally present a delicately lineolated appearance; medially on the scutellum the punctures are usually very shallow and rather indistinct; metathorax nearly smooth, shining; propodeum with the lateral and median carinae distinct, coarsely punctate between the lateral carinae, nearly smooth laterally; propodeal spiracle very small, circular; pleura shining, mostly delicately punctate; marginal vein nearly four times as long as stigmal vein, the postmarginal nearly twice as long as the stigmal; abdomen very slightly longer than thorax, ovate, pointed at tip, smooth and polished basally, faintly transversely lineolated on the posterior half. Green; head, scutellum, axillae, and postscutellum strongly bronzy; scape yellowish; rest of antennae dark brown; wings hyaline, veins pale brown; legs, including all coxae, entirely yellow; abdomen green, more or less aeneous posteriorly.

Male.—Like the female except in the following respects: Antennae with the scape shorter and broader; the pedicel stout, scarcely longer than broad at apex; the segments of the funicle elongate, twice or nearly twice as long as broad, each of the first three provided with a long slender branch, these branches subequal in length; club much broader than funicle and about one and one-half times as long as the last funicular segment; middle coxae slightly brownish at base; posterior coxae brownish black except at apex; abdomen with a pale yellow spot near base.

Type.—Cat. No. 28124, U.S.N.M.

Type locality.—Melrose Highlands, Mass.

Host.—*Apanteles melanoscelus* (Ratzeburg).

Described from nine females and seven males reared at the Gipsy Moth Laboratory; the type, allotype, and four paratypes bear Gipsy Moth Laboratory No. 11603 f 4 d; five paratypes were reared under No. 11603 a 3, and the remaining five under No. 11668 a 2.

EFFECT OF SOIL MICROORGANISMS ON PARAFFIN USED AS A COATING TO DECREASE THE INJURIOUS ACTION OF LEAD ARSENATE ON PLANT ROOTS[1]

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INTRODUCTION

Arsenate of lead mixed with soil was found by Leach (*4*)[2] to be a very effective compound for preventing the establishment of the Japanese beetle, *Popillia japonica* Newm., in the soil of certain limited areas such as cold frames. The arsenate acted as a stomach poison to the larvae. The arsenate in quantities toxic to the larvae was, however, injurious to certain plants. Particles of arsenate were coated with paraffin, various oils, fats, waxes, and other compounds in an effort to decrease the injurious action of the arsenate on these plants. Several coated lead arsenates were prepared and tested in the soil. Leach found that the arsenate coated with these materials was still as toxic to plants as the uncoated compound.

In view of the peculiar physical properties and the chemical inactivity of such materials as paraffin, it was not understood why lead arsenate coated with it was nevertheless injurious to plants. It was not unreasonable, however, to assume that some soil agent, either chemical or biological, had so modified the coating material as to liberate the arsenic. A study of the properties of paraffin led to the conclusion that the phenomenon was probably more biological than chemical. This conclusion was further strengthened when it was found that Söhngen (*7*) had described certain microorganisms which decomposed paraffin. An investigation was therefore undertaken to determine whether the soil microorganisms removed the paraffin coating from the arsenate particles.

METHOD OF STUDYING THE ACTION OF THE MICROORGANISMS

The actions of the soil microorganisms, the bacteria (including the actinomycetes), and the fungi on the paraffin-coated lead arsenate was studied in the laboratory under controlled conditions. Briefly stated, the method employed was as follows: 200 gm. of air-dried garden soil was placed in each of 140 glass jelly jars. Groups of 10 of these soil samples were mixed thoroughly with a definite quantity of either lead arsenate, lead arsenate coated with paraffin, lead arsenate coated with a mixture of paraffin and o-cresol, or paraffin finely ground with magnesium silicate.[3]

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[2] Reference is made by number (italic) to "Literature cited," p. 338.

[3] The samples of coated lead arsenate used by the writer were prepared by P. A. van der Meulen, agent of the United States Department of Agriculture.

Ten samples of untreated soil served as a control. The materials containing arsenic were added to the soil at the rate of 0.4, 0.6, 0.8 and 1.0 gm. of lead arsenate per 200 gm. of air-dried soil; the paraffin, ground with magnesium silicate, was used at the rate of 0.6 gm., the quantity contained in the highest concentration of paraffin-coated lead arsenate. When all the soils were mixed, 20 c. c. of distilled water was added to each jar of soil to bring it to the optimum moisture content of 50 per cent saturation. Five larvae were embedded in each jar of soil. At intervals of 10 days 2 jars of each kind of treated soil and 2 of untreated soil were examined to determine the effect on the larvae,[4] the numbers of bacteria and fungi,[5] and the quantities of water-soluble arsenic produced.[6]

RESULTS OF EXPERIMENTS

The results of a single series of tests, summarized in Table 1, are typical of those obtained in the several other series. When uncoated lead arsenate was added to the soil in the proportions used in this test there was an incomplete mortality of the larvae at the end of 10 days. Coating the arsenate with an inactive material such as paraffin seemed to increase slightly the rate of larvicidal action of the arsenate.

The soil fungi were not stimulated to any considerable extent by the addition of lead arsenate; in the presence of paraffin the increase in fungi was almost proportionate to the amount of paraffin. Greaves (*3*) observed that certain salts of arsenic seem to exercise a stimulating action on bacterial activity. It was also observed in the experiment here reported that the bacteria were stimulated by the arsenate used alone. Paraffin did not stimulate the bacteria appreciably.

When lead arsenate was mixed with distilled water and the supernatant liquid analyzed for water-soluble arsenic, it was found to contain 1.35 mgm. of water-soluble arsenic pentoxide for each 0.4 gm. of the arsenate. The paraffin-coated arsenates gave only 0.39 mgm. and 0.50 mgm. of water-soluble arsenic pentoxide. When the uncoated lead arsenate was mixed with soil, more soluble arsenic was produced than when the arsenate was added to distilled water. This result is in agreement with that obtained by Stewart (*8*), who concluded that "the soil solution has a greater solvent power for lead arsenate than has pure water." It was also observed that the quantity of soluble arsenic produced by the paraffin-coated arsenate in the soil had increased over the quantity found in distilled water to an extent almost equal in some cases to that of the uncoated arsenate.

[4] The larvae were removed and placed in fresh soil, and examined until their reaction could be determined.

[5] The number of bacteria (including the actinomycetes) was determined by the plate method described by Waksman and Fred (*11*). Ten grams of soil were shaken with 90 c. c. of sterile distilled water, and aliquot parts of this 1:10 dilution made up to dilutions of 1:10,000, 1:100,000, and 1:1,000,000. Five 1 c. c. samples of each of these higher dilutions were mixed thoroughly with 10 c. c. of melted sterile sodium-albuminate agar in sterile Petri dishes; after the agar had solidified the plates were incubated for 7 days at a temperature of 25° to 28° C. The colonies on each plate were counted and the number of bacteria (and actinomycetes) per gram of soil determined.

The number of fungi per gram of soil was determined by the plate method, the special medium recommended by Waksman (*10*) being used. The sterile melted special agar was inoculated in triplicate series with soil dilutions of 1:10, 1:100, and 1:1,000. The number of fungous "colonies" developing were counted after 48 hours.

[6] The quantity of water-soluble arsenic in coated and in uncoated lead arsenates was determined before they were mixed with the soil and at 10-day intervals after the mixing was done. To determine the arsenic before the mixing was done, 0.4 gm. of lead arsenate, coated or uncoated, was put into a 500 c. c. Erlenmeyer flask with 400 c. c. of distilled water, and allowed to stand for 24 hours at 25° C. The supernatant liquid was then filtered through a dry filter, and an aliquot part of the solution was analyzed according to the method recommended by the Association of Official Agricultural Chemists (*1, p. 59*). The samples of soil were tested for soluble arsenic by mixing the 200-gm. sample with 1,000 c. c. of distilled water and analyzing the solution by the same method.

TABLE 1.—*Effect of coated and uncoated lead arsenate on the fungi, bacteria, and larvae in the soil and on the quantity of water-soluble arsenic produced*

Material added to soil	Quantity of material per 200 gms. of soil	Number of organisms found [a]				Quantity of water-soluble arsenic pentoxide produced—	
		Fungi per gram of soil	Bacteria per gram of soil	Larvae		In soil	In water
				Dead	Alive		
	Gm.	*Thousands*	*Millions*			*Mgm.*	*Mgm.*
Lead arsenate	0.40	7.6	51.6	5	5	2.32	1.35
	0.60	10.3	46.0	8	2	3.87	.....
	0.80	15.3	47.5	10	0	4.02	.....
	1.00	9.3	30.5	7	3	3.90	.....
Lead arsenate 100 grams and paraffin [b] 60 grams	0.64	35.6	4.7	8	2	2.20	0.39
	0.96	54.0	2.1	9	1	3.72	.....
	1.28	27.0	2.7	10	0	1.21	.....
	1.60	120.6	3.8	10	0	1.50	.....
Lead arsenate 100 grams and paraffin [c] 59 grams, and o-cresol [c] 1 gram	0.64	.....	.....	9	1	.....	0.50
	0.96	.....	.....	10	0	.....	.....
	1.28	.....	.....	10	0	.....	.....
	1.60	.....	.....	10	0	.....	.....
Paraffin	0.60	110.0	4.5	0	10	0.42	0.00
Untreated (control)	0.00	11.3	2.0	3	12	0.42	0.00

[a] The bacteria, fungi, and water-soluble arsenic were determined 40 days after the beginning of the experiment; the effect on the larvae was determined after 10 days.
[b] As a coating.
[c] Paraffin and o-cresol mixture used as a coating.

DISCUSSION OF RESULTS OBTAINED BY OTHER INVESTIGATORS

The results of these experiments, when studied in conjunction with those obtained by other investigators, indicate that soil microorganisms, especially the soil fungi, remove the paraffin from the lead arsenate particles in the soil. A number of years ago Rahn (*5*) called attention to the presence in the soil of a species of Penicillium capable, when grown in cultural solutions containing paraffin as the only organic constituent, of utilizing paraffin in its metabolism. Gainey (*2, p. 362*) observed that an enormous development of certain species of fungi occurred parallel with the progressive disappearance of active nitrogen and of paraffin in the soil. He concluded that it was not unreasonable to assume that the enormous growth of fungi, observed where paraffin was added, utilized the paraffin as a source of carbon and of energy; also that active nitrogen which disappeared was utilized as a source of nitrogen in the metabolism of the fungi. In addition to the Penicillia, the Aspergilli, Cladosporia, Trichodermae, and the Mucorales have been isolated from the soil by Waksman (*9*) and others.

Numerous experimenters have reported that various species of Aspergillus and Penicillium produce organic acids, such as oxalic, citric, and other organic acids in the course of their metabolism. The removal of the chemically inert paraffin coating and the formation of these acids on the surface of the lead arsenate particles would tend to increase the quantity of water-soluble arsenic produced by the coated material.

Russell (*6, p. 31*) remarks that "a large number of soil bacteria have now been isolated that can decompose phenol, meta, para, and ortho cresol, and are able to use these substances as the sole sources of energy for their life processes." Söhngen (*7*) found that certain

soil bacteria also decompose and use similarly benzene, petroleum, paraffin oil, and paraffin. Soil bacteria which are able to decompose and derive their energy from naphthalene and toluene have also been isolated. The results obtained by the writer, however, seem to indicate that the bacteria are not so important as the fungi in decomposing the paraffin coating of the arsenate in the soil.

The soil microorganisms apparently have the ability to decompose the various oils, fats, waxes, and paraffin which were used by Leach (*4*) to decrease the injurious action of lead arsenate on certain plant roots. It seems futile, therefore, to attempt to protect the plants from arsenic injury by coating the poison with these materials.

SUMMARY

Coating lead arsenate with paraffin and with various oils and fats did not decrease the injurious action of the insecticide in the soil or reduce its toxic effect on plants. A study was made of the action of soil bacteria and soil fungi on lead arsenate coated with paraffin, to determine whether these organisms were responsible for this injurious action. It was found that soil fungi were stimulated by paraffin. Various species of fungi are known which are capable of utilizing paraffin in the course of their metabolism and converting it into some organic acid. The soil bacteria were less active than fungi in decomposing the paraffin coating. The conversion of the paraffin coating into organic acids may explain the injurious action of the coated insecticide in the soil.

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THE DETERMINATION OF ORGANIC MATTER IN SOILS BY MEANS OF HYDROGEN PEROXIDE[1]

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INTRODUCTION

The organic matter of soils is generally determined by converting it to carbon dioxide by means of moist and dry combustion. The carbon dioxide thus evolved is then calculated to organic matter by the use of a conventional factor, 0.471. It has been found, however, that the percentage of carbon in the organic matter of soils varies widely. Some doubt, therefore, is cast on the accuracy of the combustion methods, as applied to any particular soil. For this reason a new method which does not involve the use of an empirical factor is desirable.

Inasmuch as several investigators have reported that a large part of the soil organic matter is decomposed by hydrogen peroxide, it seemed possible that the organic matter might be determined by treating a sample of soil with hydrogen peroxide and noting the loss in weight. A treatment with hydrogen peroxide, unlike treatment by the combustion methods, would not be expected to affect the combined water content or appreciably change the weight of the inorganic material.

In testing the use of hydrogen peroxide for determining organic matter, information has been obtained regarding the decomposability of various kinds of soil organic matter; and also, concerning the accuracy of the conventional factor, 0.471, when applied to the organic matter of different soils.

REVIEW OF PREVIOUS WORK

Coppenrath, Hasenbäumer, and König (*2*)[2] and also May and Gile (*6*) have shown that the quantity of oxygen set free from the hydrogen peroxide used is roughly proportional to the quantity of organic matter in the soil.

König, Hasenbäumer, and Grossmann (*5*) found that hydrogen peroxide converted from 60 to 90 per cent of the total carbon of the soil to carbon dioxide.

Peterson (*7*), when studying the organic phosphorus of soils, was the first to determine the loss in weight of the soil material caused by the destruction of the organic matter by hydrogen peroxide. His method consisted in digesting the soil with dilute hydrogen peroxide in a covered beaker until the weight became constant. On comparing the loss in weight with the quantity of organic matter determined by combustion, he concluded that hydrogen peroxide

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[2] Reference is made by number (italic) to "Literature cited," p. 355.

destroyed about 90 per cent of the soil organic matter. Peterson did not determine whether any carbon was left in the digested residues; thus it is not known whether failure of the two methods to agree was due to incomplete oxidation or to organic matter which has a different percentage of carbon than that assumed when the conventional factor is used.

Robinson (*9*) proposed the use of hydrogen peroxide in the mechanical analysis of soils to destroy the organic matter which prevented the deflocculation of the soil particles. He observed that the destruction of the organic matter was not complete. Later he and Jones (*10*) found that cotton fiber, crude fiber from straw, etc., were not decomposed by hydrogen peroxide under their experimental conditions, whereas humus, or organic matter which had been extracted from soils by ammonia was completely decomposed. They therefore proposed the use of hydrogen peroxide to differentiate between the humified and nonhumified organic matter of the soil. The humified organic matter was determined by subtracting the ignition loss of the soil after treatment with hydrogen peroxide from the ignition loss of the untreated soil. They found that the humified organic matter varied from 3.4 to 57.5 per cent in the mineral and peat soils of Wales, and that it constituted from 42.3 to 82.3 per cent of the total organic matter, as determined by the loss on ignition. The percentage of organic matter destroyed is doubtless somewhat too low, since the loss on ignition is almost invariably greater than the organic matter contained in a soil. Robinson and Jones took precautions to minimize this error by selecting soils free from carbonates and, presumably, low in combined water, since they were of low clay content.

THE HYDROGEN PEROXIDE METHOD

In the hydrogen peroxide method the soil organic matter is determined from the loss in weight caused by digesting the soil with hydrogen peroxide. The method adopted in this investigation is described as follows:

About 1 gm. of ordinary soil or 0.2 gm. of peat was accurately weighed into a tall 250 c. c. beaker, to which 10 c. c. of water and 10 c. c. of 30 per cent hydrogen peroxide [3] were added. The beaker was covered and watched for a few minutes. If the reaction was not too vigorous,[4] the beaker was placed on a steam bath and the contents digested until no more bubbles evolved. If the reaction is too vigorous the method can not be applied without modification to the particular soil. The digestion was usually complete within 1 hour, the peats and mucks requiring more time. After the bubbles ceased no more hydrogen peroxide remained, as is shown by the permanganate test. The beaker was frequently manipulated during the digestion in order to bring the lighter particles of organic matter in contact with the solution as much as possible. After it had settled the solution was filtered through a thick and compact asbestos pad in a special Gooch crucible. Filtration is necessary to separate

[3] This hydrogen peroxide contained from 30 to 33 per cent of H_2O_2. The ash content of 0.001 per cent was disregarded. A small quantity of preservative present contained nitrogen and carbon. Suitable allowances were made for the nitrogen and carbon in some of the details of this study.

[4] A violent reaction with the evolution of heat shows the presence of manganese dioxide (MnO_2) or chromium sesquioxide (Cr_2O_3) in the soil. If the quantity of manganese dioxide is small (say less than 0.25 per cent), it can be destroyed by evaporating with a very small excess of oxalic acid, and the soil may then be treated as indicated above. If much manganese dioxide is present, this method is not applicable.

from the residue some soluble, undecomposed organic matter and ammonium salts which are always present in solution. The residue was washed a little, and the filtrate and washings were evaporated in a platinum dish, ignited and weighed. This was necessary since the weight of the ash of the soluble matter must be added to the weight of the residue.

The filtrate should be practically clear. Occasionally heavy clay soils will give a turbid or muddy filtrate. If passing the suspension through the pad a second time without allowing the pad to become dry fails to remove the suspended matter, certain corrections must be made. The weight of the ignited suspended matter can not be directly added to the weight of the residue in the Gooch crucible, since the clay loses about 10 per cent of combined water on ignition. Separate estimates of clay and soluble inorganic matter must be made, so that the ignited clay can be calculated to its weight at 110° C. This is done as follows: The filtrate is made up to a definite volume and is allowed to settle. The clear, upper half is evaporated, ignited, and weighed, giving one-half of the weight of the soluble inorganic matter. The turbid, lower half is treated in the same manner. The difference between these two weights gives the weight of ignited clay. This weight is increased by one-ninth to give the weight of the clay dried at 110° C. The corrected weight of the clay and the determined weight of the soluble, inorganic material are then added to that of the filtered residue.

The insoluble matter remaining on the pad was dried for 18 hours at 110° C. and weighed. The moisture determination in the untreated soil material was made at the same time. The difference in weight between the moisture-free material and the total weight of the dried residue plus the ash obtained from the solution was calculated as organic matter.

The concentrations and quantities of peroxide most favorable for the complete decomposition of the organic matter were selected after several trials. The results of these trials on four soils, high and low in organic matter, are given in Table 1.

TABLE 1.—*Effect of hydrogen peroxide in different concentrations on the decomposition of organic matter in different types of soil*

Types of soil	Depth at which soil sample was taken	Decomposition solution used		Organic matter decomposed
		30 per cent H_2O_2	Water	
	Inches	*C. c.*	*C. c.*	*Per cent*
Velasquez loam	12 to 32	10		12.64
Do	do	10	10	12.94
Do	do	10	50	12.73
Do	do	20	20	13.00
Do	do	40	40	13.12
Ontario loam	0 to 12	10		3.01
Do	do	10	10	2.82
Do	do	10	50	3.19
Carrington loam	do	10	10	3.93
Do	do	20	20	3.63
Do	do	40	40	3.58
Peat	46 to 70	10		87.1
Do	do	10	50	90.7
Do	do	20	20	86.6
Do	do	40	40	89.9

It is evident that accurate determinations of the dried weights of the soil and digested residues are very important. Considerable difficulty was experienced in drying the residue to constant weight in an ordinary Gooch crucible on account of the very hydroscopic nature of some of the materials. By providing the crucibles with tightly fitting ground-on covers, satisfactory results were obtained. Pyrex-glass crucibles were used.

The quantities of organic matter destroyed by hydrogen peroxide show no consistent variation with increasing quantities or concentrations of the reagent. The differences are about the same as are obtained between duplicates, although the duplicates agreed more closely in subsequent work, as the technique improved with practice. Evidently the quantity of hydrogen peroxide specified in the procedure was sufficient to produce maximum decomposition of the organic matter.

Although tests showed that nothing was to be gained by the use of different concentrations of hydrogen peroxide, it was not known whether more organic matter would be decomposed by additional treatments with the reagent. Two different soils were therefore subjected to successive treatments with 20 c. c. of the 15 per cent hydrogen peroxide used in the procedure. The results are shown in Table 2.

TABLE 2.—*Effect of successive treatments by hydrogen peroxide on quantity of organic matter decomposed in soils of different types*

Type of soil	Number of treatments with H_2O_2	Organic matter decomposed
		Per cent
Velasquez loam	1	12.64
Do.	2	12.81
Do.	3	12.65
Peat	1	87.1
Do.	2	87.8
Do.	3	90.7

The data in Table 2 show no consistent increase in the quantities of organic matter decomposed by additional treatments with hydrogen peroxide. Later work showed that, under an excessively prolonged treatment, slightly more organic, or carbonaceous matter could be decomposed by hydrogen peroxide, but the process was too tedious to be adopted as a practical method. It is thought that the procedure adopted gives as complete decomposition of the organic matter as is practicable.

COMPOSITION OF SOIL MATERIAL MADE SOLUBLE BY HYDROGEN PEROXIDE

In describing the hydrogen peroxide method it was stated that the filtrate from the peroxide digestion contained soluble matter for which corrections were made.

About half of the matter made soluble was volatile at a dull-red heat. Part of the volatile matter was obviously organic, but its nature was not determined. König, Hasenbäumer, and Grossmann (*5*) found that formic and acetic acids were formed under similar

conditions. Ammonium salts also formed part of the volatile matter; in fact analyses of several of the salt solutions showed that practically all the soil nitrogen was converted into ammonium salts.

It is clear that the soluble, volatile matter should constitute a part of the organic matter indicated by the loss in weight of the material when treated with hydrogen peroxide. Thus, the weight of the residue from the peroxide digestion is not corrected for the soluble volatile matter. The ash remaining after the volatile matter is driven off, however, should be added to the weight of the residue. This correction for the ash, always made, ranged from 0.11 to 2.04 per cent, and was important in most cases. The ash does not seem to result from the solution of the soil inorganic matter, since the quantity of ash is not proportional to the quantity of hydrogen peroxide used, to the volume of the solution, or to the time of digestion. Evidently the ash comes from the organic matter which is decomposed, since different soils usually give quantities of ash proportional to the quantities of organic matter present. Furthermore, the ash yielded by different quantities of a particular soil is proportional to the quantity used.

The partial composition of the soluble inorganic matter present in the solution resulting from the digestion of three different soil materials with hydrogen peroxide is given in Table 3.

TABLE 3.—*Partial composition of inorganic matter in soils of different types made soluble by hydrogen peroxide (expressed as a percentage of the weight of soil)*

Type of soil	SiO_2	Al_2O_3+ Fe_2O_3	CaO	MgO	MnO
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Carrington loam	0.112	0.048	0.064	0.021	0.002
Hagerstown loam	.038	.022	.388	.044	.040
Velasquez loam	.073	.015	.168	.013	N. D.

Although the soluble inorganic matter of soils contains sulphates and chlorides also, the quantities of these elements were not determined. Evidently the constituents of the ash vary considerably in different soils. Lime is obviously one of the chief constituents. König, Hasenbäumer, and Grossmann (*5*) also found lime to be the chief soluble constituent under similar conditions. In some soils manganese may be the most important constituent of the ash. This was found to be so in the case of the Blakely and Amito loams.

It seems likely that most of the soluble inorganic matter has its origin in the destruction of the organic matter of the soil. In a sense it might be considered a part of the organic matter. However, it is left in the soil when the organic matter decays, and in all combustion methods it is neglected. It seemed best, therefore, to deduct the weight of the soluble, inorganic matter from the entire loss in weight caused by the digestion of the organic matter by hydrogen peroxide.

EXTENT TO WHICH CERTAIN KINDS OF ORGANIC MATTER ARE DECOMPOSED BY HYDROGEN PEROXIDE

Before determining the decomposability of the total soil organic matter it seemed best to study the action of hydrogen peroxide on some simpler organic materials, especially in view of the fact that

Robinson and Jones (*10*) reported that unhumified organic matter, such as cotton fiber and crude fiber, were not attacked by peroxide.

Preliminary tests made in the laboratory showed that the conclusion of Robinson and Jones regarding the nondecomposability of unhumified organic matter is true only under certain conditions. It was found that 15 per cent hydrogen peroxide had practically no effect on cotton fiber and crude fiber, provided they were digested in the absence of other material. However, when the digestion was carried out in the presence of soil both the cotton fiber and crude fiber were destroyed almost quantitatively.

Inasmuch as other investigators have found that all the soil organic matter is not decomposed by hydrogen peroxide, such material as charcoal, coal, and graphite were included in the substances tested, since they are likely to occur in soils and presumably are difficult to oxidize. Since the presence of soil material has such a marked effect on the decomposability of cotton fiber and crude fiber, the tests of the decomposability of various kinds of carbonaceous substances were made on samples mixed with a portion of the Orangeburg fine sandy loam subsoil. The proportion of organic matter decomposed was determined from the loss in weight of the sample in some cases, and in other cases, by measuring the carbon content before and after treatment. The results are given in Table 4.

TABLE 4.—*Decomposition of various kinds of carbonaceous matter by 15 per cent hydrogen peroxide in the presence of soil*

Kind of carbonaceous matter	Quantity decomposed
	Per cent
Coal, anthracite (100-mesh screen)	10.2
Coal, bituminous (100-mesh screen)	7.8
Coal, cannel (100-mesh screen)	14.2
Charcoal in contact with soil but a short time [a] (100-mesh screen)	29.8
Charcoallike particles separated from soil [b] (100-mesh screen)	89.4
Cotton fiber	99.6
Crude fiber from wheat straw	98.4
Graphite, natural (100-mesh screen)	0.0
Graphite schist separated from soil	0.0
Humus, extracted by ammonia	100.0
Lignite (100-mesh screen)	98.7
Lubricating oil	66.4
Peat, mosslike	99.5
Peat, dark brown	97.0

[a] Charcoal particles were separated from a cultivated soil where a large brush pile of hardwood had been burned about nine months before the charcoal was gathered.

[b] Composite of several very small samples of charcoallike particles, separated from a number of soils and subsoils by Schreiner and Brown (*11*).

Table 4 shows that coals and graphite, in particular, were hardly attacked, and that charcoals were decomposed in varying degrees. More than half of a lubricating oil was destroyed, and the other substances were decomposed almost quantitatively.

It is certain that in the method adopted hydrogen peroxide can not be used to distinguish humified and nonhumified organic matter. Brown peat, mosslike peat, cotton fiber and crude fiber were almost completely decomposed under the conditions of this study. It is true that Robinson and Jones used weaker hydrogen peroxide and allowed a shorter time for digestion; but it is believed that the discrepancy between their results and those given in Table 4 is due

chiefly to the fact that they used hydrogen peroxide alone, whereas in the experiments described in this paper soil material, acting as a catalyzer, was always present during the digestion.

From these data it would be expected that the procedure adopted would result in the decomposition of all except the most resistant carbonaceous matter found in soils. The results for charcoal tend to show that different charcoals vary considerably in decomposability.

RESULTS OF ANALYSES OF DIFFERENT SOILS

A satisfactory procedure having been established, samples of material from a number of different soil types were analyzed for organic matter by the peroxide method.

Samples were selected from several soil types which are very high and several which are very low in organic matter. Three soil colloids and several very clayey subsoils were also selected with a view to studying the possible effects of colloid surfaces and of a high percentage of combined water on the accuracy of the method. To test the possible effect of the action of hydrogen peroxide on soil mineral matter, materials from several very deep subsoils low in organic matter were included. It was thought that if any oxidation of the mineral matter took place it would be greatest in the deep subsoil materials where natural oxidation is not so pronounced as in surface layers. Duplicate determinations were made on all samples. After the material had been treated with hydrogen peroxide, the residues were analyzed for undecomposed organic matter by the combustion method,[5] and also for carbonates.[6] These results are given in Table 5.

The results for organic matter, in Table 5, have not been corrected for the carbonates found in the residues. In each case the combustible matter found in the residue has been calculated to carbon, since it seemed likely that it contained a high percentage of carbon. All quantities are expressed as percentages of the weight of the original material used, dried at 110° C.

It is apparent, from columns 3 and 4 in Table 5, that the duplicate results obtained by the peroxide method agree about as well as those of other constituents, obtained by ordinary methods of soil analysis. To get closely agreeing duplicates it is necessary to observe extreme care in getting the dried weights. The sample should be weighed from a weighing bottle, and the Gooch crucible should be covered promptly just before transferring it from the drying oven to the desiccator.

The carbon dioxide of the carbonates in the residue evidently results from the action of hydrogen peroxide on the organic matter. The carbon dioxide, shown in the last column of Table 5, should properly be added to the organic matter found, since the residue is too heavy by just that quantity. However, it is apparent that such a correction would not be of any importance ordinarily. In 15 of the 26 materials analyzed this correction would be less than 0.15 per

[5] An electric combustion furnace giving a maximum temperature of 1,100° C. was used. With a quartz combustion tube it was possible to heat the sample nearly to the maximum temperature, thus insuring complete combustion of graphite and other difficultly combustible carbon of low combustibility. The mixed gases from the furnace were dried by phosphorus pentoxide both before entering and in the carbon dioxide absorption apparatus.

[6] Only one soil contained carbonates before treatment. A small quantity of carbonates is formed by the action of the hydrogen peroxide on the soil.

cent of the dry weight of the sample analyzed. The values given for organic matter in columns 3 and 4 of Table 5 have not been corrected for carbonates.

TABLE 5.—*Organic matter in soils of various types as determined by the hydrogen peroxide method and the undecomposed carbon and the carbonates in the residues*

Type of soil material	Depth at which soil sample was taken	Quantity of organic matter as determined by decomposition with H_2O_2 (duplicate results)		Quantity of combustible matter calculated as carbon, in residue from H_2O_2 treatment	Carbonates in the residues from the H_2O_2 treatment (calculated as CO_2)
	Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Amito loam, Mississippi	0–12	2.35	2.39	0.48	0.06
Do	12–36	1.63	1.73	.37	.02
Blakeley loam, Georgia	0–12	2.14	2.24	.23	.24
Carrington loam, Iowa	0–12	[1] 3.59	[1] 3.59	.30	.00
Carrington loam colloid, Iowa	0–12	10.17	10.08	.41	.06
Cukre clay, Nicaragua, Central America	0–5	8.89	8.89	.67	.16
Do	5–60	2.12	2.04	.28	.17
Hagerstown loam, Pennsylvania	0–8	4.78	4.67	1.90	.19
Houston black clay, Texas	0–12	3.39	3.49	.60	.20
Mosslike peat, Florida	0–8	95.60	95.50	.25	.02
Muck, Indiana	0–6	22.45	22.36	1.29	.75
Do	6–12	12.17	12.38	.60	.33
Ontario loam, New York	0–12	[1] 3.00	[1] 3.00	.06	.05
Orangeburg fine sandy loam, Mississippi	10–36	.30	.41	.06	.00
Orangeburg fine sandy loam colloid, Mississippi	0–10	3.62	3.58	.38	.03
Lenox stony loam, Massachusetts	0–6	4.83	4.83	.50	.04
Peat, Florida	46–70	90.70	89.90	1.45	.37
Palmar clay, Colombia, South America	0–14	6.67	6.76	.26	.15
Do	14–60	1.06	.95	.07	.06
Do	60–72	.84	.74	.10	.06
Do	72–96	.60	.61	.06	.02
Do	96–120	.51	.51	.04	.00
Ranger loam, Georgia	0–10	2.00	1.80	.33	.00
Sharkey clay, Mississippi	0–4	3.98	3.72	.44	.21
Velasquez loam, Guatemala, Central America	12–32	[2] 12.90	[2] 12.90	.73	.27
Wabash silt loam colloid, Nebraska	0–15	4.60	4.59	.52	.09

[1] Average of 6 determinations. [2] Average of 12 determinations.

The carbonaceous matter not oxidized bears no constant relation to the total quantity of organic matter present. In a few cases it is inconsiderable when calculated as carbon, amounting to less than 5 per cent of the total organic matter. In most of the materials, however, the undecomposed carbonaceous matter is considerable. It is evident, therefore, that the hydrogen peroxide method does not give an accurate determination of the total organic matter, that is, of the total quantity of carbon, or carbon compounds exclusive of carbonates.

NATURE OF THE UNDECOMPOSED ORGANIC MATTER

Since considerable quantities of organic matter or some substances containing carbon are not decomposed by the hydrogen peroxide treatment, the identification of these carbon-containing substances in the residues is important. If the residue after digestion contains only a definite kind of organic matter, the hydrogen peroxide method could be used to differentiate the two kinds of organic matter present in soils, and also to determine the quantity of the decomposable portion.

The quantity of organic matter left undecomposed is not dependent primarily on the state of subdivision, since the organic residue from the mosslike peat is less than the organic residues from the three soil colloids.

General knowledge concerning the soils treated was of little value in determining the nature of the undecomposed organic matter, except in the case of the sample of Hagerstown loam. This sample, taken from the "charcoal plot" of the Pennsylvania Experiment Station, was known to contain charcoal; and it will be noted in Table 5 that the quantity of undecomposed carbon is exceptionally large in this soil (*13*). The Ranger loam and the Lenox stony loam are peculiar soils in that they are derived from graphite schist. In samples of these soils the quantities of undecomposed carbonaceous matter are not large; hence it is improbable that graphite constitutes any considerable part of the carbon in the undecomposed residues of the other soil materials.

The preliminary work on the decomposition of various organic materials indicated that graphite, coal, and certain kinds of charcoal, if present in soils, would be found in the part not attacked by hydrogen peroxide. However, it is not to be expected that graphite, widely distributed in small quantities, would occur in anything more than traces in most soils. Coal particles would be restricted to coal-bearing regions. Because of the prevalence of forest fires, charcoal might be present in almost all soils. The decomposability of charcoals, however, is evidently variable.

Schreiner and Brown (*11*) found coallike and charcoallike particles in all of 34 soils examined by them, and also in samples from a number of deep subsoils. They point out that numerous forest fires furnish an ample source of charcoal, but add that it is not known how long the charcoal persists in the soil. White and Holben (*14*) showed that charcoal had persisted in the charcoal plot of the Pennsylvania experimental plots for more than 68 years. This particular plot was once the site of a charcoal furnace, and the soil on it now contains about 4 per cent of carbon. White and Holben found that charcoal in small quantities was a general constitutent of all the Pennsylvania plots, and they consider charcoal a general, though small, constituent of soils in the eastern United States.

The residues from the hydrogen peroxide treatment were examined microscopically for evidence as to the nature of the undecomposed organic matter. Occasionally particles resembling roots were observed, but these particles seemed to be mere outlines and entirely different in appearance from roots not treated with hydrogen peroxide. In the Hagerstown loam, black particles, evidently charcoal, could be seen. The black particles gather on the sides of the beaker during the digestion. Charcoal can be distinguished by a test, devised by J. G. Smith of this laboratory, which consists of drawing a spatula firmly over a small quantity of soil material which has been placed on a clean, white sheet of paper. Charcoal gives a characteristic black streak. This may be confirmed by burning the black particles and by microscopic examination. White and Holben (*14*) recommend testing the soil for charcoal by stirring the soil in water; the charcoal particles will then rise to the surface.

Apparently there are forms of carbonaceous matter in the digested residues which do not possess the characteristics of charcoal, coal, or graphite. The residues from the peat and muck soil contained small quantities of a waxy substance which yielded dense, white fumes on burning. The odor and the fumes given off resembled those of paraffin. None of the other residues gave off white fumes on

burning. The fact that they blackened momentarily, indicated that a small quantity of organic matter was distributed through the residues. This momentary blackening may have been due to a trace of soluble organic matter which was not thoroughly removed by washing, since König, Hasenbäumer, and Grossmann (*5*) have found that acetic and formic acids are formed by the action of hydrogen peroxide on soil organic matter.

It is desirable to know the percentage of carbon in the carbonaceous matter of the undecomposed residues. It was found that further oxidation of the carbonaceous matter could be obtained by prolonged boiling of the residues in a closed flask with hydrogen peroxide. The flask was connected with a Knorr carbon dioxide apparatus and the carbon dioxide given off was weighed. Ten cubic centimeters of the 30 per cent peroxide was added and the contents of the flask were gently heated and finally boiled until no more carbon dioxide was given off, the process requiring several hours. Ten cubic centimeters more of peroxide was added, and this procedure was repeated until no more carbon dioxide could be obtained from the residues, 6 to 10 treatments being required. The boiled residue was then collected on a Gooch crucible, dried, and weighed, and the loss in weight noted. From the loss in weight of the sample and the weight of the carbon dioxide evolved the percentage of carbon in the carbonaceous matter destroyed was calculated. The carbon remaining after the prolonged boiling with hydrogen peroxide was determined by subtracting the quantity of carbon thus oxidized from the quantity of carbon present in the residue obtained in the standard method. These determinations, together with the carbon content of the residues from the first hydrogen peroxide treatment, are given in Table 6. Unless otherwise noted, the percentages are calculated on the weight of the oven-dried material rather than on the weight of the residue.

TABLE 6.—*Effect of prolonged boiling with hydrogen peroxide on the carbonaceous matter that remains after soils are treated by the standard peroxide method*

Type of soil or colloid	Quantity of carbon left after treatment by the standard peroxide method	Quantity of carbonaceous matter destroyed by prolonged boiling with hydrogen peroxide	Percentage of carbon in the carbonaceous material destroyed by prolonged boiling with hydrogen peroxide	Quantity of carbon left after prolonged boiling with hydrogen peroxide
	Per cent	*Per cent*		*Per cent*
Hagerstown loam	1.90	2.01	90	0.09
Carrington loam colloid	.41	.22	95	.20
Muck (surface layer)	1.29	.52	73	.91

The quantities of carbon in the residues before and after prolonged boiling show that practically all the carbonaceous matter in the Hagerstown loam was decomposed by this treatment and that only from one-half to one-third of that contained in the Carrington loam colloid and muck was digested. The matter decomposed, being highly carbonaceous, is probably charcoal, while that unattacked is, presumably, not charcoal. These assumptions are supported by the fact that the carbon in the sample of Hagerstown loam, a soil material containing a large quantity of charcoal, was practically all

oxidized. Furthermore, a test showed that finely ground, nearly fresh charcoal was completely decomposed by prolonged boiling with peroxide.

It would seem from the above determinations that charcoal, or some material very high in carbon, usually constitutes a part of the carbonaceous matter that is left in the first treatment with hydrogen peroxide. Other more resistant carbonaceous substances may also be present. It is not known whether this resistant matter is high or low in carbon. The residues obtained in the analyses of Hagerstown loam, Houston black clay, and Sharkey clay by the standard method were found to contain insufficient nitrogen to give a test. It is probable, therefore, that the resistant matter contains no nitrogen.

It is not possible to state definitely the nature of the carbonaceous matter which resists decomposition by hydrogen peroxide in the adopted procedure. It is quite certain, however, that all organic matter of the nature of humus, crude fiber, and other cellulose material is decomposed. This is shown in the preliminary tests of these materials, and also by the fact that the residues from the peat and mosslike peat were very small. Preliminary tests with the adopted method indicate also a partial decomposition of charcoal or coallike material. From the microscopic examination it seems that a considerable part of the undecomposed organic matter in most of the materials examined may be charcoal, and graphite in two of the samples. In two cases a paraffinlike substance in the residues was indicated by ignition. Prolonged boiling with hydrogen peroxide also indicated that charcoal or a substance very high in carbon contained practically all the carbon of the undecomposed residue in one sample, and from one-half to one-third of the carbon in the residues of two other soils. Apparently the organic residues obtained from different soils contain different forms of carbon, and more than one kind of carbonaceous matter may be present in a single residue. In general, however, charcoal seems to be the most common carbonaceous constituent.

COMPARISON OF THE HYDROGEN PEROXIDE AND COMBUSTION METHODS OF DETERMINING ORGANIC MATTER

A comparison of the quantity of organic matter destroyed by the hydrogen peroxide method with the total organic matter determined by combustion is important. Although the former method is not accurate in determining the total organic matter in soils, since considerable carbon is found in the residues, the latter method is likewise inaccurate because of the uncertainty of the use of the factor for a particular soil. The hydrogen peroxide method has an advantage in that it gives a direct determination of the weight of the organic matter destroyed; and, since the quantity of undecomposed carbonaceous matter is small in many cases, it would seem that this method might give results as near the correct value as would the combustion methods.

Combustion determinations of organic matter were made on all the soils and colloids used in this investigation. The results of these combustion determinations are given in Table 7. In this table the organic matter determined by the hydrogen peroxide method is corrected for the carbonates present in the residues. The quantity of carbonaceous matter not decomposed by the peroxide is calculated

as carbon. The sum of the organic matter determined by hydrogen peroxide plus the quantity of carbon found in the residues is given for comparison with the combustion and hydrogen peroxide results. All percentages are calculated on the dry weight of the untreated material.

TABLE 7.—*Comparison of organic matter of different soils as determined by the combustion method and the hydrogen peroxide method*

Type of soil and colloid	Depth at which soil sample was taken	Quantity of organic matter determined by combustion ($CO_2 \times 0.471$)	Quantity of organic matter determined by hydrogen peroxide	Carbonaceous matter obtained from residue of peroxide digestion calculated as carbon	Organic matter obtained by hydrogen peroxide method plus the carbon in the residue	Organic matter as determined by combustion method compared with organic matter determined by hydrogen peroxide method plus the carbon in the residue
	Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Amito loam	0–12	3.34	2.43	0.48	2.91	0.43
Do	12–36	2.30	1.70	.37	2.07	.23
Blakeley loam	0–12	2.91	2.43	.23	2.66	.25
Carrington loam	0–12	4.00	3.59	.30	3.89	.11
Carrington loam colloid	0–12	11.57	10.19	.41	10.60	.97
Cukre clay	0–5	9.56	9.05	.67	9.72	−.16
Do	5–60	2.49	2.25	.28	2.53	−.04
Hagerstown loam	0–8	9.22	4.92	1.90	6.82	2.40
Houston black clay	0–12	4.20	3.64	.60	4.24	−.04
Lenox stony loam	0–7	5.22	4.87	.50	5.37	−.15
Mosslike peat	0–8	85.60	95.57	.28	95.85	−10.25
Muck	0–6	24.47	23.16	1.29	24.45	.02
Do	6–12	14.09	12.61	.60	13.21	.88
Ontario loam	0–12	3.07	3.05	.06	3.11	−.04
Orangeburg fine sandy loam	10–36	.45	.36	.06	.42	.03
Orangeburg fine sandy loam colloid	0–10	4.28	3.63	.38	4.01	.27
Peat, from Florida	46–70	97.49	90.67	1.45	92.12	5.37
Palmar clay	0–14	7.38	6.87	.27	7.14	.24
Do	14–60	.87	1.06	.07	1.13	−.26
Do	60–72	.73	.85	.10	.95	−.22
Do	72–96	.68	.63	.06	.69	−.01
Do	96–120	.44	.51	.04	.55	−.11
Ranger loam	0–10	2.24	1.90	.32	2.22	.02
Sharkey clay	0–4	4.19	4.01	.44	4.45	−.26
Velasquez loam	12–32	15.06	13.17	.73	13.90	1.16
Wabash silt loam colloid	0–15	5.54	4.69	.50	5.19	.35

A comparison of columns 3 and 4 of Table 7 shows that the combustion method gives higher results than the hydrogen peroxide method in 22 of the 26 determinations, the differences being considerable in most cases. In 4 cases the hydrogen peroxide results exceed those obtained by the combustion method.

In some cases the ignition loss indicates which result is nearer the true value. In any case the ignition loss should exceed the quantity of organic matter, since it includes the combined water of the inorganic colloidal material and part of the sulphur and chlorine. The Florida peat has an ignition loss of 95.6 per cent. In this case the ignition loss must be nearly the correct value for organic matter, since very little water can be combined with inorganic material. Here the combustion value for organic matter, though obviously a little high, is more nearly correct than the hydrogen peroxide value. The ignition loss of the mosslike peat, 96.5 per cent, shows that, in

this case, the hydrogen peroxide value is the more accurate. Moreover, the fact that there is very little carbon in the undecomposed residues from this peat is another indication that the hydrogen peroxide value is very nearly correct. The muck subsoil has an ignition loss of 13.24 per cent, which is probably the correct value for organic matter, since the muck appears to be a mixture of quartz sand and organic matter, little or no water being combined with the organic material. The combustion value here is certainly too high, and the peroxide value is too low. In the case of the Hagerstown loam, the difference between the percentage of organic matter lost through ignition (9.52 per cent) and the percentage obtained by combustion (9.22 per cent) is only 0.30 per cent. This soil would contain ordinarily about 2.50 per cent of water combined with the inorganic colloidal material, since it contains 25 per cent of colloidal matter which averages 10 per cent of combined water. The ignition loss indicated in this case is 7.02 per cent of organic matter. The combustion value seems to be too high, therefore, by a difference of 2.20 per cent, and the peroxide value too low by a difference of 2.10 per cent. The ignition losses would seem to indicate that in one determination the combustion value is the more accurate, that in another determination the peroxide value is the more accurate, and that in two cases there is no choice between the two methods.

The correct value for organic matter is probably very close to the sum of the organic matter obtained by the hydrogen peroxide method, and the carbon in the residue; but it is probably slightly greater than this sum, since the carbonaceous matter in the residue is presumably not pure carbon. In any case this sum would represent the minimum quantity of organic matter present and would be only slightly less than the true value, as the quantity of carbon in the residue is small. The variation of the hydrogen peroxide values and the combustion values from this assumed correct value are shown in columns 5 and 7, respectively, of Table 7.

It is to be noted that in 11 cases there is no choice between the combustion and hydrogen peroxide methods, the values given by these methods differing by 0.16 or less from the assumed correct values. In 8 instances the combustion values are closer than the peroxide values, the average variation of the peroxide values and the maximum variation being 0.55 per cent, and 1.29 per cent, respectively, of the weight of the soil. In 7 determinations the peroxide results are nearer the assumed correct values. The combustion values in these 7 cases differ by an average of 3.04 per cent, and show a maximum deviation of 10.25 per cent.

It is apparent that the peroxide method gives about as accurate results, although usually low, as the combustion method. In the case of somewhat unusual soil materials also the results may be considerably more accurate.

ACCURACY WITH WHICH CONVENTIONAL FACTOR OF 0.471 APPLIES TO ORGANIC MATTER DESTROYED BY HYDROGEN PEROXIDE

It has just been shown that some of the combustion values for organic matter are more or less incorrect. Obviously the reason for this is that the factor used for calculating the carbon dioxide to organic matter does not correctly represent the composition of the organic matter of certain soils.

The use of the factor, 0.471, was first proposed by Wolff (*15*), in 1864. He found that there was, on the average, 58 per cent of carbon in the humus extracted from soils and in artificial humus preparations. To get the weight of organic matter, therefore, he recommended multiplying the quantity of carbon dioxide evolved on oxidation by the factor, 0.471. This factor has been used for the determination of organic matter in soils ever since.

It is clear that a factor derived from the average composition of soil organic matter may be quite inaccurate when applied to any particular soil, although it may correctly represent the average composition of the organic matter of a number of soils. From a large number of subsequent determinations, however, it is apparent that there may be considerably less than 58 per cent of carbon in the average soil organic matter. For instance, the average percentage of carbon in the humus extract of 40 soils, as given by Eggertz (*3*), Cameron and Breazeale (*1*), and Fraps and Hamner (*4*) is 45.6. This evidence is not conclusive, since the humus extract is only a part of the organic matter, and its composition may not be representative of all the organic matter present. Snyder (*12*) and Read and Ridgell (*8*) determined the carbon in the entire organic matter in 81 samples of soil. The average percentage of carbon in these 81 samples was 49.4.

The above averages indicate that the factor, 0.471, may be too low for the average soil. Further evidence regarding the accuracy of this factor is evidently desirable. The data in Table 7 bear on this point. By the use of these data, it is possible to calculate the percentage of carbon in most of the organic matter in each of the 26 samples examined. The hydrogen peroxide destroyed almost all the organic matter in many of these soil materials. The weight of the organic matter destroyed in each case is known, and the percentage of carbon in the organic matter may be calculated by subtracting the carbon in the residue from the total carbon, as determined by combustion, and dividing the remainder by the organic matter decomposed by hydrogen peroxide. The proportions of carbon calculated in this way are given in column 7 of Table 8.

The figures given in Table 8 show that the percentage of carbon in the organic matter decomposed by hydrogen peroxide varies considerably, ranging from 37.5 to 70.1, with an average of 54.8 per cent. Of the 26 samples tested, 5 samples (Nos. 15, 19, 20, 21, and 22) show determinations of slight dependability on account of the low organic content; and one soil, the Hagerstown loam, gives an abnormally high carbon percentage, on account of the artificial addition of charcoal. The remaining 20 samples cover a wide range of soils, including as they do, 2 peats, 2 mucks, and 3 soil colloids. Excluding Nos. 8, 15, 19, 20, 21, and 22, for the reasons noted above, the average carbon content for the remaining 20 is 56.3 per cent, with a minimum of 49.8 per cent and a maximum of 61.9 per cent.

When all the results of digestion by hydrogen peroxide are considered, the average percentage of carbon is considerably higher than the averages reported by investigators subsequent to Wolff. In the selected list, the average is still higher, being fairly close to the 58 per cent required to make Wolff's (*15*) conventional factor applicable.

TABLE 8.—*Quantity of carbon in the organic matter of various types of soil destroyed by hydrogen peroxide*

Sample No.	Type of soil and colloid	Depth at which soil sample was taken	Quantity of organic matter as determined by decomposition with hydrogen peroxide	Quantity of combustible matter calculated as carbon in residue from H_2O_2 treatment	Quantity of carbon in total organic matter of soil [1]	Quantity of carbon in organic matter destroyed by hydrogen peroxide
		Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Amito loam	0–12	2.43	0.48	66.5	59.9
2	do	12–36	1.70	.37	64.4	57.0
3	Blakeley loam	0–12	2.43	.23	63.2	59.9
4	Carrington loam	0–12	3.59	.30	59.6	56.2
5	Carrington loam colloid	0–12	10.19	.41	63.3	61.9
6	Cukre clay	0–5	9.05	.67	57.0	53.9
7	do	5–60	2.25	.28	59.4	54.1
8	Hagerstown loam	0	4.92	1.90	78.4	70.1
9	Houston black clay	0–12	3.64	.60	57.5	50.5
10	Lenox stony loam	0–7	4.87	.50	55.3	50.6
11	Mosslike peat	0	95.57	.28	51.8	51.9
12	Muck	0–6	23.16	1.29	58.0	55.7
13	do	6–12	12.61	.60	61.8	60.1
14	Ontario loam	6–12	3.05	.06	57.2	56.5
15	Orangeburg fine sandy loam	10–36	.36	.06	62.1	56.4
16	Orangeburg fine sandy loam colloid	0–10	3.63	.38	61.9	58.0
17	Peat	46–70	90.67	1.45	61.3	60.8
18	Palmar clay	0–14	6.87	.27	59.8	58.3
19	Do	14–60	1.06	.07	44.6	41.0
20	Do	60–72	.85	.10	44.5	37.5
21	Do	72–96	.63	.06	57.4	53.4
22	Do	96–120	.51	.04	46.4	42.1
23	Ranger loam	0–10	1.90	.32	58.5	51.3
24	Sharkey clay	0–4	4.01	.44	54.6	49.8
25	Velasquez loam	12–32	13.17	.73	62.1	60.8
26	Wabash silt loam colloid	0–15	4.69	.50	61.9	57.9
	Average, all samples				58.6	54.8
	Average, excluding Nos. 8, 15, 19, 20, 21, and 22				59.7	56.3

[1] It is assumed that the organic matter not decomposed by H_2O_2 is carbon.

Although the preceding calculation applies to a larger part of the total organic matter than the calculations of Wolff, Eggertz, and others who have worked on humus extracts, it does not apply completely to the total organic matter, since a small quantity is not decomposed by hydrogen peroxide. There is some evidence that the organic matter not decomposed by hydrogen peroxide is higher in carbon than that which is decomposed. If this is so, it would raise the percentage of carbon in the total organic matter, in each case to a figure slightly higher than that expressed by the figure in column 6, Table 8. On the assumption that the organic matter in the residues is all carbon, the percentage of carbon in the entire organic matter of the soil was calculated. The results are given in column 7 of Table 8. The average carbon content for the 20 selected soils is 59.7 per cent. Doubtless the true average percentage for the total organic matter lies at some point between 56.3 and 59.7 per cent of carbon. It can thus be seen that the conventional factor, 0.471, corresponding to 58 per cent carbon, applies very well to the average organic matter in the soil types used in this study.

In individual cases the application of the factor leads to comparatively large errors, the maximum being 10 per cent. However, these errors occurred in soils so very high in organic matter that the combustion method is not ordinarily applied to them. The Hagerstown loam is an exception, because the large quantity of charcoal present in it is responsible for considerable error. From these results, it

seems reasonable to conclude that occasionally large errors may result from the use of the conventional factor. In general, however, this factor seems to represent the composition of organic matter rather accurately.

LIMITATIONS AND ADVANTAGES OF THE HYDROGEN PEROXIDE METHOD

From the point of view of manipulation the hydrogen peroxide method is satisfactory. The agreement of duplicates is good. The basic idea is simple, though fully as much care must be given to details of procedure as in the combustion method. The hydrogen peroxide method offers no advantages so far as rapidity of determination is concerned, over the combustion method, except when a large number of analyses are to be made.

The hydrogen peroxide method is not applicable to soils which contain more than 2 or 3 per cent of calcium carbonate or more than a trace of manganese dioxide or chromium sesquioxide. Soils containing chromium sesquioxide are rare; and soils containing more than a trace of manganese dioxide are, as a rule, confined to the tropical or subtropical latitudes.

As a means of determining the total organic matter in soils, the hydrogen peroxide method shows little, if any, inferiority to the combustion method. The peroxide method always gives low results; but the errors are small in most cases. The combustion method, on the other hand, sometimes gives too high or too low results, and occasionally the errors are large.

Although hydrogen peroxide does not completely decompose the organic matter in the soil, there is no good evidence that it determines any clearly defined kind of organic matter. The so-called "humus" is entirely destroyed; but since peat, crude fiber, and other cellulose materials are likewise decomposed by hydrogen peroxide, the method obviously can not be taken as a means for determining the humus in soils. Neither can the part of the soil which is not decomposed by peroxide be regarded as containing all the charcoal originally present, for under some conditions considerable charcoal is decomposed.

It is presumed that the carbonaceous matter remaining in the residues from the peroxide treatment is inert and hard to oxidize by biological processes. Probably this unattacked carbonaceous matter is not so important in soil fertility as the organic matter which is attacked by hydrogen peroxide. On the other hand, the peroxide method obviously can not be used to determine the readily available organic matter.

For routine analysis, the hydrogen peroxide method offers no advantage over the combustion method. However, in investigations where an accurate determination of the organic matter is necessary, as in the determination of the water which is combined with the inorganic material of soils, a combination of the hydrogen peroxide and combustion methods offers distinct advantages. If the carbon in the residue from the hydrogen peroxide digestion were determined by combustion and then added to the organic matter determined by hydrogen peroxide, the sum would be a more accurate figure for the total organic matter than the value obtained by any of the present methods.

SUMMARY

A report is given of the possibility of determining the organic matter in soils by the loss in weight caused by digestion with hydrogen peroxide, and a method for this determination is described.

Several forms of organic matter, including cellulose, humus, and lignite, are almost completely decomposed by hydrogen peroxide in the presence of soil. Graphite is unattacked and charcoal and coal are partially decomposed.

Determinations made by the hydrogen peroxide method on a variety of soils and colloids containing from 0.42 to 95.85 per cent of organic matter show that practically all the organic matter is decomposed in some soils, and that in other soils considerable organic matter is unattacked.

The nature of the carbonaceous matter resisting decomposition by hydrogen peroxide is uncertain. In some soils, charcoal forms a major part of the undecomposed organic matter, but other organic matter may also be present.

Although the hydrogen peroxide method gives low results, it is, on the whole, about as accurate as the combustion method, and may be considerably more accurate in special cases.

The method gives some indication concerning the percentage of carbon in soil organic matter. In the 26 samples analyzed, the organic matter averaged about 58 per cent carbon. This is the proportion of carbon required by the conventional factor, 0.471, used for calculating the carbon dioxide evolved by combustion to organic matter. Individual cases show considerable variation from this average of 58 per cent.

The hydrogen peroxide method is not applicable to soils high in calcium carbonate, manganese dioxide, or chromium sesquioxide.

Apparently the hydrogen peroxide method does not determine any clearly defined kind of soil organic matter. It decomposes more than the so-called "humus," and is therefore not a method to be used in determining the humified organic matter in the soil.

For routine analysis, the hydrogen peroxide method offers no advantages over the combustion methods. It should, however, prove useful in investigations, since this method, when it is supplemented by a combustion determination of carbon in undecomposed residues, should give the most accurate value for soil organic matter.

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DIPLODIA EAR-ROT DISEASE OF CORN[1]

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INTRODUCTION

The most common and destructive ear-rot disease of corn is caused by the fungus *Diplodia zeae* (Schw.) Lév. This disease is found in almost every locality in which corn is grown.

The first definite description of this fungus was given by Heald, Wilcox, and Pool (*7*),[2] and a more careful and exhaustive study of the disease was made later by Burrill and Barrett (*3*) working in Illinois. They showed that the ears were invaded either from the butt or the tip and that ears in the milk stage were most susceptible to infection; that the stalks were relatively immune to attack; and that the fruiting bodies of the fungus were produced freely on old stalks. Burrill and Barrett showed further that the spores readily survived the winter and were blown for considerable distances by the wind during the following spring. The two last-named writers estimated that 90 per cent of the ear-rot losses in Illinois were caused by this one disease.

Less than a decade ago *Diplodia zeae* was universally regarded as distinctly an ear-rot problem. Since the advent of the corn root-rot, stalk-rot, and ear-rot investigations, however, this fungus has been rather generally referred to in combination with several Fusaria as a causal agent for corn ear rot, stalk rot, and root rot. Thus in the report of the Ohio Agricultural Experiment Station for 1921 (*16*) appears the statement that "during the past three years careful investigation has been made for the purpose of determining the predominating cause of the corn root-rot disease in Ohio. This has been found to be the fungus *Diplodia zeae*, more generally known as a cause of corn dry rot or mold. This disease is present generally throughout the State, yet is most serious in the western and southwestern sections." Durrell (*4*) and Durrell and Porter (*5*) report that this fungus attacks the ears, shanks, and joints of the corn plant freely, but that it does not cause the roots to decay nor, under Iowa conditions, is it active in attacking the seedlings. They state further that following infection of the seed the stand obtained from this seed is considerably reduced numerically and the plants are weakened. Holbert, Burlison, Koehler, Woodworth, and Dungan (*8*), discussed *Diplodia zeae* as a cause of root rot, ear rot, and seedling blight of corn. They found that planting diseased seed gave a reduced stand and that the surviving plants made an irregular growth. Corn grown from infected seed was found to have more leaning stalks and stalks on the ground, but not more broken stalks.

Koehler, Dungan, and Holbert (*10*) state that "infection of seed corn with this organism [*Diplodia zeae*] results in a greater reduction

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[2] Reference is made by number (italic) to "Literature cited," p. 370.

in yield than infection with any other organisms herein reported [*Fusarium moniliforme Cephalosporium acremonium*, and *Gibberella saubinetii*]."

At the present time *Diplodia zeae* is referred to not merely as cause of ear rot but also as a factor in the root, stalk, and ear rot situation. It is said to cause losses immediately as the result of molding ears, and in the long run, also, through the infection of seed and the consequent reduction in crop yield.

The present investigation was begun in 1921 in Ohio, where it was pursued two years. The writer continued the work in New York during the years 1923, 1924, and 1925. While many phases of the problem have been touched upon incidentally, the problem of seed infection and the effect of this infection on the germination, development, yield, and incidence of ear rot have been the major objectives.

RESULTS OF EXPERIMENTS

Field observations in Ohio in 1921 showed that the ear rot caused by *Diplodia zeae* was most prevalent in the western part of the State where corn was a major crop. A survey of the seed situation in this section was made during the winter with the idea of discovering the prevalence of Diplodia infection. The results of testing 2,900 ears for 82 farmers located in six counties showed 24.2 per cent of the ears infected—an exceptionally high average. Most of these infected seed ears did not show marked external signs of disease. This led to the supposition that they had been infected late in the summer. Through the following experiment more definite information was obtained as to the times during which natural infection took place in the fall and late summer of 1921.

A large field of corn growing on the farm of the Ohio Agricultural Experiment Station, Columbus, Ohio, was visited at intervals, beginning August 31, when the ears were in the milk stage. Each time approximately 60 ears were removed at random, taken to the laboratory, and placed in racks to dry. The corn in this field was cut September 27; hence all selections after that date were made from the the shocks. The results of a careful test of these various lots of ears made early in the winter are shown in Table 1.

TABLE 1.—*Occurrence of natural ear-rot infection on corn picked at different periods ranging from August 31 to December 1, 1921, on farm of Ohio Agricultural Experiment Station*

Date ears were picked	Number of ears picked	Percentage of ears infected	Percentage of kernels per infected ear		
			Healthy	Diseased	Dead
Aug. 31	60	6.6	34.38	31.25	34.37
Sept. 20	51	9.8	32.50	20.00	47.50
Oct. 10	64	18.7	46.87	31.26	21.87
Oct. 28	67	19.1	45.63	23.62	30.75
Dec. 1	60	40.0	29.25	38.50	32.25

These data show that some infection had already occurred by August 31 while the corn was still in the milk stage, and that the percentage of ears infected increased rapidly up to December 1, the date of last sampling. Especially notable is the increase in the percentage of infected ears which took place during November while the corn was standing in the shock. This increase, however, does not necessarily prove the occurrence of new infections during this period. It is quite possible that conditions within the shock were favorable for the growth of the fungus, and infections that were present on the ear, shank, or stalk at the time the corn was cut were, under these favorable conditions, transferred to the corn ears. In any case, however, the importance of selecting and drying seed corn early in the fall is emphasized. Further proof that the rot fungus readily invades matured ears is available as the result of the following inoculation experiments.

Two lots of 25 ears each were inoculated August 31, when the corn was in the milk stage, and two more on September 20, when the corn was almost ripe.

The inoculation was done by wounding each ear in the shank and pressing into each wound a bit of clover stem on which the fungus had been cultured. The results of the inoculations are shown in Table 2.

TABLE 2.—*Results of early and late infection with ear rot of two lots of corn of 25 ears each on August 31 and September 20, 1921, Ohio Agricultural Experiment Station*

Date inoculated	Percentage of ears infected	Percentage of kernels per infected ear		
		Healthy	Diseased	Dead
Aug. 31	100.0	0	7.5	92.5
Sept. 20	84.0	53.2	34.3	12.5

These ears were tested late in the year after the corn was perfectly dry. The lots inoculated while the corn was in the milk stage were badly molded, light in weight, with cobs showing evident decay. The ears were such as would be thrown out at husking time as worthless. The late-inoculated ears, on the other hand, showed no cob rot or surface growth of the fungus. The cobs of these ears looked clean and reasonably free from discoloration. The results of these tests indicate that the badly molded ears found at husking time represent early infections, while the less severely diseased ears found in seed corn represent late infections that were soon checked by the drying out of the corn.

The environmental factors favoring infection and rotting of the ears have not been determined under controlled conditions. Observations, however, indicate that abundant moisture during the early fall is highly favorable. After ear infection has once occurred the loss of moisture incidental to the drying out of the ears is commonly the factor which brings to a close the activity of the fungus. Corn-

rot fungus in ears stored at temperatures favoring fungus activity may, after the ears are completely air-dry, become inactive. Since no moisture determinations were made in the experiments previously referred to, the following data from Kiesselbach and Ratcliff (*9*) are given to indicate the approximate moisture content of corn that is thoroughly air-dried:

	Percentage moisture in embryo	Percentage moisture in endosperm
New corn (field selected):		
Before fully dry	31. 1	24. 1
When air-dry	10. 5	12. 9
Old corn:		
When air-dry	7. 91	7. 6
Embedded 24 hours in moist soil	21. 37	15. 62
Embedded 48 hours in moist soil	36. 81	20. 83

As would be expected, the activity of the fungus in diseased ears very gradually decreases as the ears become drier, until the point is reached when further advances can not be detected by the germination test.

The following records of a test made in the fall of 1921 illustrate the gradual manner in which fungal growth ceases as the infected ears of corn become dry. Mature ears of dent corn harvested September 27 were placed in a warm attic and allowed to remain there until October 7, at which time they appeared to be dry. Tests were made and records taken on that date and again one month later. In making the two tests, kernels side by side were removed from the ears. The records of the two tests are shown in Table 3.

TABLE 3.—*Progress of Diplodia infection as influenced by drying of ears, Ohio Agricultural Experimental Station, 1921*

Date of test	Percentage of kernels per ear		
	Healthy	Diseased	Dead
Oct. 7	56. 7	43. 3	0
Nov. 4	53. 2	34. 3	12. 5

It will be observed that in those ears which were rapidly approaching the air-dried condition the rate of fungal advance was very slow. All the ears used in the test were inoculated September 20, so that during the 2½ weeks immediately following inoculation 34.3 per cent of the kernels were invaded, while during the next 4 weeks the percentage of infected kernels was increased by only 3.5 per cent. It is apparent that early field selection of seed corn and immediate drying are very effective in reducing the amount of Diplodia infection.

HOW CORN-EAR INFECTION OCCURS

The spores of the fungus causing corn-ear rot are carried through the air. The tip of the ear surrounded by the partly dead and dried silk remains would seem to offer a favorable point of entrance for these spores, especially since the corn-ear worm, which attacks at this point, might logically be supposed to open the way for ear-mold infection. Durrell (*4*), however, states that under Iowa conditions in 1921 the

majority of infections began at the butts of the ears. The writer's observations over a period of years are fully in accord with this conclusion. Thus, for example, a record was kept of some 1,700 ears comprising 170 exhibits at a corn show. The highest percentage of infection in any exhibit was 70 and the lowest 0. The average for the entire show was 13.3 per cent. Owing to the fact that these ears had been selected especially for exhibit this average was much lower than the seed corn average in the district for the year. Of the entire number of diseased ears, 89.9 per cent were infected at the butt, 2.7 per cent at the tip, and 7.4 per cent at points between the butt and the tip. These figures, coupled with other observations, indicate that this fungus has little need for the assistance of the ear worm. The fact that seed ears are invaded by a number of fungi in addition to Diplodia is well known. Manns and Adams (*11*) have attempted to determine the relative prevalence of these organisms. Their data show that two Fusaria were very prevalent everywhere. These organisms, *Gibberella saubinetii* and *Fusarium moniliforme*, were very common in the seed corn mentioned above. It seemed worth while, therefore, to determine the immediate damage caused by the Fusaria as compared to Diplodia. It was not thought advisable to attempt to distinguish between the two species of Fusaria. Ears infected with Diplodia averaged 8.8 per cent of the kernels dead, while ears infected with the Fusarium averaged 2.3 per cent of the kernels dead. Hence Diplodia infection is much more likely to result in the death of the kernel than Fusaria infection. This is explained by the fact that the Diplodia attacks the embryo by preference, while the Fusaria invade the starchy portions of the kernels.

DIPLODIA ZEAE AS A CAUSE OF SEEDLING BLIGHT

This fungus is common in seed corn. Many kernels in infected ears are invaded but not killed. When these diseased but living kernels are planted they soon absorb sufficient moisture to permit fungal activity. It should be borne in mind, however, that whereas in the fall moisture is the factor that limits the development of the ear-rot phase of the disease, temperature appears as the factor limiting the development of the seedling blight phase in the spring. Thus, if diseased seed is planted in warm soil, the fungus quickly grows out from the kernel and attacks the mesocotyl of the seedling. Since the mesocotyl tissues are very susceptible, decay progresses rapidly as far as the primary node. Beginning at the primary node, the tissues are resistant; hence the advance of the rot is sharply checked at this point. The secondary roots thrown out at the primary node are not attacked. If the diseased seedling lives until these are established, it is safe. Under temperature conditions favorable for the disease some seedlings are killed before the secondary roots develop.

The relationship of temperature to the development of seedling blight was tested as follows: Four tanks were filled with water. Two of them were kept at a temperature of 15° to 18° C. by permitting cold water to trickle into them. The other two were maintained at a temperature of 29° to 31° C. by heating them. Two 3-gallon crocks filled with sterilized soil were placed in each tank. Four similar crocks were placed on a bench in the greenhouse, where the temperature ranged from 21° to 24° C. Sowings of diseased and healthy ker-

nels were made at uniform depths in all of these crocks and the resulting plants were allowed to grow for three weeks. At the end of this period all plants were carefully washed free from the soil and the results tabulated. Table 4 is a record of the results of several experiments of the character described.

TABLE 4.—*Effect of soil temperature on the development of seedling root rot*

Kind of seed planted	Temperature (°C.)	Number of kernels sown	Percentage of seed not sprouted	Percentage of seedlings with roots—		
				Healthy	Severely rotted	Slightly rotted
Healthy (control)	15–18	40	2.5	97.5	0	0
Do	21–24	40	2.5	97.5	0	0
Do	29–31	40	7.5	92.5	0	0
100 per cent diseased	15–18	24	58.3	37.5	0	4.2
Do	21–24	24	37.5	8.3	50.0	4.2
Do	29–31	24	16.7	25.0	45.8	12.5
Diseased and healthy, half and half	15–18	32	21.8	65.7	9.4	3.1
Do	21–24	32	40.7	21.8	34.4	3.1
Do	29–31	32	21.9	25.0	31.2	21.9

Table 4 shows that the coolest temperature was unfavorable and the two warmer soil temperatures favorable for the development of the seedling root rot. It seems safe to conclude that there will be little rotting at temperatures below 20° C.

Repeated plantings of diseased seed in the field have resulted in very slight development of seedling root rot. This is apparently accounted for by the fact that temperatures of field soil during the seedling stage are generally below 20° C. A consistent feature of the field plantings has been the poor stand resulting from diseased seed. Thus healthy seed germinating on test 100 per cent would give a field stand of not less than 95 per cent, while diseased seed germinating on test 60 per cent would be likely to give a field stand of 40 to 45 per cent. Table 4 shows that the diseased seed produced a poor stand at the coolest temperature. Holbert, Burlison, et al. (*8*), state that Diplodia-diseased seed produced a poor stand in cool weather and a much better stand in warm weather. The fact that numbers of kernels, seriously attacked and weakened by the Diplodia fungus, lack the strength to push through the soil is the reason assigned for the poor stand with cool temperatures.

RELATION OF DIPLODIA SEED INFECTION TO LOSS OF STAND DURING THE GROWING SEASON

Up to this point the discussion has considered (1) the problem of infection of seed ears with Diplodia in the fall, the period of activity of the fungus in the ears, and the relation of the fungus to moisture content, and (2) the effect of this seed-borne infection on germination and stand in the field during the seedling stage. There remains for consideration the effect of corn ear rot on stand later in the season.

The literature on corn root rot contains numerous references to weak and stunted plants and also to the death of plants. During the course of the experiments covered by this discussion counts were made as soon as the plants were well up in the spring and again late

in the summer when the ears were maturing. The results for each year were the same, which the 1924 data as recorded in Table 5 will serve to illustrate.

TABLE 5.—*Effect of seed infection on stand of corn throughout summer, at New York (Geneva) Agricultural Experiment Station, 1924*

Plot No.	Stand on June 25 from—		Stand on November 25 from—	
	Healthy seed	Diseased seed	Healthy seed	Diseased seed
	Stalks	*Stalks*	*Stalks*	*Stalks*
1	55	55	52	51
2	54	54	51	51
3	56	56	56	54
4	58	58	50	58
5	56	56	55	53
6	55	55	54	52
7	58	58	56	57
8	58	58	55	58
9	60	60	60	56
10	58	59	57	57
Total loss in stand			22	22

That the loss in stand happened to total exactly the same for the diseased and healthy seed was, of course, merely a coincidence. In other tests the loss from healthy seed sometimes exceeded the loss from diseased seed, and vice versa. Never, however, has there been any indication that the planting of Diplodia-infected seed resulted in any loss of stand during the summer months.

The experiments established beyond reasonable doubt that Diplodia seed infection does not destroy plants except in the very early seedling stages, and that the plants growing from diseased seeds which survive the early attack of the fungus are as likely to mature as plants from healthy seeds. Another fact apparently well established is that the secondary root system is not susceptible to attack. Durrell (*4*) placed large quantities of inoculum directly under a number of growing plants without effect. The writer has repeatedly planted healthy seed and scattered rice culture of the fungus thickly in the hills about the seed without any perceptible effect. As a more severe test of the resistance of plants grown from healthy seed to corn root rot, healthy seeds were sown in crocks of sterilized soil which had been inoculated with the fungus in pure culture. One series of these crocks was placed in the greenhouse. The roots of plants in the diseased soil showed slight decay at a number of points. This experiment was made in 1922. In another experiment, made in 1923, healthy seed was grown under field conditions. In this experiment the roots from diseased and healthy soil appeared equally bright and clean. Under field conditions, therefore, the fungus does not cause a root rot. Valleau, Karraker, and Johnson (*15*) report that numerous isolations from decaying corn roots failed to yield *Diplodia zeae* in any case.

RELATION OF SEED INFECTION TO EAR-ROT INFECTION

The question of the possible relationship of seed-borne infection to later outbreaks of ear rot is one of great importance. Does the planting of infected seed result in a crop of infected ears? Smith

and Hedges (*13*) have reported that the fungus may spread to the stalk from the roots and have advanced the theory that ear infection may result in this manner. For four years, however, the writer has planted diseased and healthy seed side by side without securing any consistent variations in the percentages of infected ears. If ear infection ever results from the spread of the fungus from the roots, this occurs so seldom as to be a negligible factor. This was likewise the conclusion of Van der Bĳl (*1*).

There still remains, however, the possibility of a definite relation between seed infection and later development of ear rot. The ability of the fungus to grow as far up as the base of the stalk has been proved. This would be sufficient to bring it to the surface of the ground. It is possible that fruiting bodies and spores produced by the fungus at this stage may be distributed by wind and rain to the ears. On this assumption, plantings of diseased and healthy seed made side by side would be exposed equally to infection. This may be the possibility that Tryon (*14*) expresses as follows: "This 'tainted' seed will not, should it germinate, as only happens when the infection is light, directly give rise to a plant in whose system, consequently, the disease will develop; but, failing to do so, it may, when placed under circumstances favorable to growth of the fungus, yield the Diplodia spores that may prove a source of eventually infecting many other maize plants that otherwise would remain healthy; and 'dead seed' will, with utmost likelihood, do so."

To determine the possible relationship between seed infection by corn root rot and the later development of ear rot, plantings of diseased and healthy seed were made in plots well separated from one another during the three years 1923, 1924, and 1925. The ears from each planting were harvested separately and carefully tested during the winter. In no case was there evidence that the sowing of diseased seed had any influence on the quantity of ear rot in the resulting crop. The corn grown from diseased seed was quite as free from ear rot as the corn grown from healthy seed.

The low incidence of ear rot in the corn grown from these plantings of diseased and healthy seed is worthy of note. In the 1925 experiment only 2 out of 960 ears tested showed Diplodia infection. Corn had not been grown for three years or more previously in the soils used for these tests, and the fodder had been removed in the fall from the few cornfields in the near vicinity. The failure of ear-rot infection under these conditions points to the efficiency of rotation and sanitation control measures. In their original work Burrill and Barrett (*3*) emphasized the value of rotation and removal of the fodder from old fields. They showed that the spores were distributed freely and for considerable distances by the wind and that the old stalks from the previous year's crop left scattered over the surface of the ground were infection sources.

EFFECT OF SEED INFECTION ON THE VIGOR OF STALK GROWTH AND ON THE YIELD OF EAR CORN

In a test made in 1921 diseased and healthy ears were carefully selected. This corn was shelled separately, giving in one case seed 100 per cent healthy and in the other seed of which portions were either dead, diseased but living, or healthy. This second lot will be

referred to as the diseased seed. Sowings were made in adjacent rows. The results of this test are recorded in Table 6.

TABLE 6.—*Effect of seed infection on corn yield per plant at Ohio Agricultural Experiment Station, 1921*

Kind of seed	Number of kernels planted	Number of stalks obtained	Total yield in pounds	Yield per stalk in pounds
Healthy	200	186	98.7	0.531
Diseased	200	108	59.4	.550

There was no visible difference in the vigor of growth of the corn produced by the two lots of seed. The slight difference in yield favoring the diseased seed is fully accounted for by the difference in stand.

In 1923 a large number of ears were tested very carefully and diseased and healthy lots selected. In several tests the healthy ears showed no evidence of fungal infection. The diseased ears showed infection with Diplodia only. These two lots of ears were shelled separately. The healthy seed was then divided into halves. One half was inoculated with pure cultures of the fungus grown on rice; the other half was held untreated. These three lots of seed—healthy, inoculated, and diseased—were then used to plant two series of plots. Series 1 was sown on May 8 and series 2 on June 5. Each plot consisted of a single row of 10 hills, 3 plants to the hill, and the hills 3 feet apart. Sufficient seed was sown in each hill to permit later thinning to a uniform stand. The planting and thinning were both done by the writer, and in every case great care was taken to insure absolute impartiality in removing the plants.

These plots were kept under close observation the entire summer. No differences in vigor of growth were observed. The plants from diseased seed, from healthy seed, and from healthy seed inoculated, grew equally well.

In the fall yields from the plots located in the two outside rows of the experimental block were disregarded. The corn in other plots, however, was husked and the yields of ear corn recorded, as shown in Table 7.

TABLE 7.—*Effect of seed infection on corn yields at New York (Geneva) Agricultural Experiment Station, 1923*

Kind of seed	Number of stalks		Number of bearing stalks		Weight of ears		Weight of corn per bearing stalk		Average weight per bearing stalk
	First planting	Second planting	First planting	Second planting	First planting	Second planting	First planting	Second planting	
					Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*
Healthy	29	30	28	29	246	357	8.8	12.3	10.67
Do	29	30	28	29	298	320	10.6	11.0	
Healthy inoculated	30	30	27	27	288	318	10.7	11.8	10.9
Do	30	30	27	25	321	234	11.9	9.4	
Diseased	30	30	30	28	337	284	11.2	10.1	10.4
Do	30	30	29	28	282	298	9.7	10.6	

From the data presented in Table 7, it may be seen that the yields per bearing stalk for diseased, healthy, and healthy inoculated seed are very close together, with the averages slightly favoring the healthy seed as compared to the diseased seed.

This experiment was repeated on a larger scale in 1924. Preparations for the experiment were begun in the fall of 1923, when two lots of bright, clean ears were selected from each of two ear-to-row plantings. A number of these ears were inoculated as follows: Cavities were scooped out at the butt ends and the pure cultures of Diplodia fungus placed therein. These inoculated ears were then stood on moist cotton in Mason jars. They were held under these conditions one week and then removed and allowed to dry. A germination test after they were fairly dry showed that in most of these inoculated ears the fungus had penetrated about half the length of the cob. By this procedure it was possible to secure diseased and healthy seed in sufficient quantity and from the same ear strain. Any ears which showed upon testing chance infections of other fungi were discarded. As in the previous experiments, healthy seed was also inoculated by mixing with it just prior to planting pure cultures of the fungus. The land chosen for this test was exceptionally level and uniform. It had not been cropped to corn for several years.

The seed was sown on May 28. On June 25 all plots were thinned to an even stand. The plots were kept under constant observation throughout the season, but no differences in growth could be detected. In November the corn was harvested and the ear weights shown in Table 8 were recorded.

TABLE 8.—*Relation of seed infection to total corn yield and yield per bearing stalk at New York (Geneva) Agricultural Experiment Station, 1924*

Kind of seed planted	Plot No.	Number of stalks matured	Number of barren stalks	Total weight of ears	Weight of ears per bearing stalk
				Ounces	*Ounces*
Diseased	1	52	1	334	6.5
Do	2	51	6	266	5.9
Do	3	56	2	340	6.3
Do	4	50	8	256	6.1
Do	5	55	7	315	6.6
Average		52.8	4.8		6.28
Healthy, inoculated	1	54	6	299	6.2
Do	2	53	14	224	5.7
Do	3	56	11	274	6.1
Do	4	55	19	222	6.2
Do	5	56	19	225	6.1
Average		54.8	13.8		6.06
Healthy seed	1	55	7	282	5.9
Do	2	51	15	233	6.5
Do	3	54	13	249	6.1
Do	4	53	9	275	6.2
Do	5	53	17	208	5.8
Average		53.2	12.2		6.10

The figures for yield per bearing stalk fall very close together, with the results slightly in favor of the diseased seed. There was an increase of barren stalks in the healthy inoculated plantings as

compared with the diseased plantings. These differences appear to have been associated with imperceptible soil differences, since the buffer plantings, all sown with the same seed, showed the same variations.

The three-year comparison of diseased and healthy seed made in the experiments already discussed, as well as the observations of others, fail to show that Diplodia-infected seed noticeably influenced the vigor of growth or reduces the yield. It should be emphazied, however, that this refers not to plantings of 100 per cent diseased seed as compared with plantings of healthy seed, but to plantings of the usual composite obtained from diseased ears, which includes both healthy and infected kernels.

RELATION OF SEED INFECTION TO INCIDENCE OF LEANING OR BROKEN, BARREN, OR PREMATURELY DEAD STALKS, OR BROKEN SHANKS

During the course of these experiments notes were made for the purpose of discovering if possible any correlation between seed infection and the incidence of leaning or broken stalks, barren stalks, prematurely dead stalks, or broken shanks. No such correlation was found. This is quite in line with Durrell's observations (*4*). Koehler, Dungan and Holbert (*10*), on the other hand, report more leaning stalks from Diplodia-infected seed than from healthy seed. The differences they discovered, however, were small.

DISCUSSION OF RESULTS

Many of the results obtained in these experiments merely corroborate the findings of previous investigators. It was discovered that the fungus invades ears with the greatest ease, usually entering at the butt end. Ears which are infected as the corn approached maturity do not suffer conspicuous injury, and hence are likely to be selected as seed. Under favorable environmental conditions seedling root rot resulted from plantings of infected seed. In the field, however, soil conditions at corn-planting time were such that this difficulty was not encountered. The only immediately noticeable result from the planting of diseased seed was the poor stand.

The possible relationship of seed infection to ear infection later was carefully investigated. An argument often advanced against the planting of diseased seed corn is that this practice will result in an increased percentage of unsound ears. This theory is based on the supposition that there is a correlation between seed infection and the later occurrence of ear rot. These experiments covered by this discussion have failed to demonstrate a connection between Diplodia seed infection and Diplodia ear rot. On the contrary, the evidence leads to the conclusion that the farmer who plants diseased seed is not increasing his chances of suffering loss from this form of ear rot at least.

The question of the effect of seed infection on the later growth of the crop and on the yield is also very important. Durrell and Porter (*5*) state that "in addition to the reduction in stand, it was very noticeable that the rows planted with Diplodia dry rot infected seed had a high percentage of weak, stunted plants, which either died or were so weakened that they reduced the yield." Their

figures, however, do not show any very serious influence of this sort. Thus in their 1922 tests the stand reduction from the planting of the diseased seed was 8.6 per cent and the reduction in the yield 7.7 per cent. In 1923 the stand reduction was 12.1 per cent and the yield reduction 12.2 per cent. Koehler, Dungan, and Holbert (*10*) report a stand decrease of 36.3 per cent and a yield decrease of 32.4 per cent from sowing diseased seed. These authors state that "infection of seed corn with this organism results in a greater reduction in yield than infection with any other organisms herein reported." The other organisms referred to by these authors were *Fusarium moniliforme*, *Cephalosporium acremonium*, and *Gibberella saubinetii*. In all of these experiments, however, the reduction in yield is attributed to two factors—(1) the decreased stand and (2) the weakened condition of the plants. The effect of decreased stand on the yield, however, need not be allowed to intrude, since Kiesselbach and Ratcliff (*9*) have worked this out to a mathematical certainty. The real question involved is how seriously is the vigor of stand reduced as the result of sowing diseased seed.

In considering this question, attention must be called to the experimental procedure followed in the Illinois tests. In this work a number of diseased ears was selected, from each of which all healthy seed and all dead seed were discarded, leaving only the kernels that were infected but alive. The diseased seed which would be planted by a farmer, however, would be quite different. To begin with, it is not likely that more than 30 per cent of the farmer's seed ears would be infected. The average infected seed ear would test about one-eighth dead, three-eighths alive but diseased, and four-eighths healthy kernels. Adding to the 70 per cent of the farmer's healthy seed the four-eighths, or 15 per cent, portion of his diseased ears that tested healthy gives a total of 85 per cent of his shelled seed corn that is actually healthy. Of the diseased 15 per cent remaining, one-eighth, or 3.75 per cent, is dead. This leaves a total of only 11.25 per cent that is diseased but living and hence capable of producing the weakened stalks.

Actually, many of these diseased seeds when sown in the field will not produce any plants. Assuming that every diseased seed will grow and produce a stalk, the ratio of stalks from healthy seed to that of stalks from diseased seed will be more than 8 to 1. Under actual field conditions, therefore, every plant produced by a Diplodia-infected seed would be surrounded by plants from healthy seed, whereas in the Illinois test artificial restrictions were resorted to in order to insure as nearly as possible a stand of plants from diseased seed only. It is easy to see how under these conditions a measurable reduction in yield owing to reduced plant vigor could result. Under natural conditions it is not at all difficult to understand how slight weakness on the part of one-ninth of the stand would be largely compensated for by the other eight-ninths. It is on this basis that the results obtained in the experiments in which there was no measurable decrease in yield from the planting of seed from diseased ears, when this seed was sown thick enough to give a 100 per cent stand, are explained.

In these experiments the great majority of stalks in the diseased seed plots were produced by normal, healthy kernels. This is accounted for by the fact that many of the diseased kernels were

dead at the time of planting and many others lacked the strength to reach the soil surface. Futhermore, the reduction in yield obtained from planting only diseased seed need not be ascribed to a pathological activity continuing through the life of the plant, but may be fully accounted for on the basis of the immediate destructive activity of the fungus in the seed. The effect of this activity would be to weaken the seed, and there are good reasons for believing that, in the case of corn, weakening of the seed will reduce the yield. Thus Brown (*2*) has shown that mechanically multilated seed when planted gives a poor stand and weak-growing plants which yield less than the controls grown from perfect seed. He found that the reduction in yield was proportionate to the amount of mutilation. Schmidt (*12*) and others, working with sweet corn, have shown marked yield differences favoring large as compared to small seed. In the experiments here detailed 100 per cent Diplodia seed infection, has been produced through smearing the shelled seed with pure cultures of the fungus just prior to planting. This method of infecting seed eliminates the weakening effect on the seed due to fungal invasion. Seed so inoculated has repeatedly grown and yielded as well as the uninoculated healthy seed.

In this connection it is interesting to note Edgerton's conclusion (*6*) concerning *Cephalosporium acremonium* and *Fusarium moniliforme*. As the result of several years of field testing, he states that diseased seed corn gives poor stands, but that the plants from poor ears seemingly produce as much corn as the plants from good ears, and that no material differences in yield result if equal stands are obtained.

Any attempt to list the Diplodia ear-rot disease as a serious seed-borne trouble must take into consideration that the important diseases included in this class possess characteristics as follows:

First. Grain smuts, of which loose smut of wheat is a good example, cause a twofold loss. These diseases destroy the heads or ears of grain crops, thus reducing the value of the crops and in some cases reducing the quality of the crop as well. They infect the maturing seed, also, thus carrying their destruction into the next year. The relation of seed infection to head or ear destruction, and hence to seed infection and the infection of the crop of the subsequent season, is clearly drawn.

Second. Numerous vegetable crop diseases, such as black rot and black leg of the crucifers, blight and anthracnose of beans, may pass the winter in a very few seeds and yet be present in sufficient quantity to cause 100 per cent crop infection the following year. This is accounted for by the phenomenal ability of the organisms causing these diseases to multiply and spread from plant to plant during the growing season.

Diplodia when present as seed infection does not spread to the stalks and destroy the ears, as in the case with the smuts, nor does it serve as the original source from which the disease spreads from plant to plant, as is the case with vegetable crop diseases. It is the writer's opinion that Burrill and Barrett (*3*) covered the important phases of the Diplodia situation rather completely when they proved this fungus to be the cause of a widely destructive ear-

rot disease and demonstrated its ability to overwinter freely on old corn refuse and to spread from thence to the ears the following season.

SUMMARY

The fungus *Diplodia zeae* is the cause of a destructive corn ear rot. Infections, in the great majority of cases, begin at the butt, and the fungus apparently grows into the cob by way of the shank.

Under ordinary field conditions the ears are susceptible to infection almost from the time they are formed until they are mature; to cause extensive molding, however, fungal invasion must occur while the ears are immature.

The fungus remains active within the infected ears until they have become thoroughly dry.

Ears infected late in the season are not visibly molded, and hence are often selected for seed. When planted, seed from such ears gives a poor stand and a correspondingly reduced yield as compared to seed from healthy ears. In the experiments with which this discussion is concerned however, no differences in yield resulted if the seed from diseased ears was sown thick enough to compensate for the reduced germination.

Comparative plantings of diseased and healthy seed repeated over a three-year period showed no increase in ear rot due to sowing diseased seed.

Diseased and healthy seed sown on land not cropped to corn for three years or more and well removed from fields containing old stalks and remains of previous season crops have both produced corn almost 100 per cent free from Diplodia ear-rot infection.

Where Diplodia infection is anticipated, early removal of seed ears will avoid a large part of the infection. Prompt drying will check the spread of the fungus in cases where ear infection has already occurred.

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MEASURES OF PERSISTENCY OF LACTATION[1]

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INTRODUCTION

In agricultural colleges it is frequently taught that cows differ markedly in persistency of lactation, or the degree with which the milk flow is maintained as the stage of lactation advances after calving. The dairy breeds are considered superior to the beef breeds and unimproved cattle in this particular; and as between the individual cows of any one dairy breed there are differences in persistency.

Similar remarks may be made with reference to yearly yield. In this case there is abundant quantitative data to substantiate the teaching. Many determinations of the annual milk and fat yields of cows have been made, which give for each individual the year's production in the form of a definite figure. This is the important figure which measures the worth of the cow from a cost-accounting standpoint. From a biological standpoint we know that the yearly production is the resultant of a complex of forces or factors. It is desirable for the purpose of biological analysis to break up the total production into its component parts, so far as possible.

It seems logical to regard the yield of milk for any definite period of time as the result of the rate of milk secretion during that period. Milk secretion is initiated by the bearing of young, and the mammary gland reaches a maximum potential capacity for milk secretion periodically or recurrently with the close of gestation. From a biological standpoint, one may therefore deal with the rate of milk secretion during the period following calving. In the present state of development of the dairy cow, the function of lactation usually continues uninterruptedly for 10 months or even much longer, depending upon the recurrence of conception. Obviously, a suitable measure of the rate of milk secretion may give a measure of production which is independent of the length of time covered by the production record. Such a measure of the rate of milk secretion will indicate its initial value at calving and its change with advance in lactation. This latter is the characteristic commonly referred to as persistency of lactation, in the numerical evaluation of which the writer is especially concerned in this paper.

MEASURES OF PERSISTENCY

The earliest numerical measure of persistency which has come to the writer's attention is that of Sturtevant (*8*)[2] which expresses the decrease in milk yield each month as a percentage of the milk yield of the preceding month. This idea has been used by various investigators who followed Sturtevant. Carlyle and Woll (*2*) employed as a measure of persistency the ratio $100\,\frac{a-b}{a}$ where a is the yield for a certain early week in the lactation period, and b is the yield for a certain late week in the lactation period. This principle has also

[1] Received for publication Nov. 26, 1926; issued April, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 383.

been followed by other investigators. McCandlish et al. (*6*) have used the method of expressing each month's milk yield as a percentage of the first month's yield and comparing, graphically, the slopes of the curves thus obtained. This method does not give a numerical value to persistency.

There is evident a certain kind of relationship between the three measures just described. They have been used as cited only in comparisons of groups of cows and not in detailed studies of individuals.

Obviously, what is needed is a numerical expression representing persistency for each individual cow so that the character may be studied quantitatively by statistical methods and brought into line with our knowledge of other quantitative characters. Sanders (*7*) has presented the first detailed study of this sort. As his basic measure of persistency he used the ratio of the lactation milk yield to the maximum day's yield. This measure is affected greatly by the length of the lactation, which in turn is affected by the time from calving to conception (service period). Sanders accordingly applies a correction for service period.

Turner (*9*) has recently proposed two measures of persistency. The first is essentially that used by Sturtevant and expresses the average ratio of each month's yield (except the first) to that of the preceding month's yield. The second is similar to that of Sanders and expresses the ratio of the twelve months' yield to the yield of the maximum month, from which ratio may be derived the supposedly corresponding ratio of the first method. The first method when applied to the irregular data of individual records is mathematically unsound as a measure of the general tendency of the record and may be dismissed from further consideration here.[3] The principle of the second method will be considered in some detail below.

The published records of the American Guernsey Cattle Club offer inviting material for the study of persistency. In studying the question of persistency in this laboratory, the writer has felt that it was desirable to obtain for each record under consideration an equation which would represent as accurately as possible the rate of milk secretion through the course of lactation undisturbed by pregnancy. Daily energy yield in terms of 4 per cent milk (*4*) is considered in preference to direct use of the raw data of milk or fat yield as published by calendar months.

Consideration was given first to the straight line equation

$$\frac{dy}{dt} = C - bt \qquad (1)$$

in which y represents yield; t, time from calving as origin; $\frac{dy}{dt}$, the rate of yield; C and b are constants, C representing the initial rate of yield and b the rate of *absolute* decrease in rate of yield. The constant b is used as a measure of persistency.

Equation (1) was abandoned in favor of the exponential equation

$$\frac{dy}{dt} = ae^{-kt} \qquad (2)$$

in which y, t, and $\frac{dy}{dt}$ have the same meaning as in (1); a is a constant

[3] It is not desired to imply that this method may not give a *practically* useful measure, but it tends to give too high values, especially when monthly yields are irregular.

representing the initial rate of yield; e is the base of natural logarithms; and k is a constant representing the rate of decrease in the rate of yield as *proportional* to the rate of yield of the moment. The constant k is used as a measure of persistency.[4] Exponential expressions have been found of wide use in the description of both physical and biological phenomena. So far as the writer is aware, this type of equation was first used in description and interpretation of the lactation curve by Brody et al. (*1*).

DETERMINATION OF THE CONSTANTS

In a more or less preliminary investigation (*3*) of some of the Guernsey records the writer has used a special graphic method of determining the a and k constants of equation (2) for each record. If the observations fall in anything like a regular order, the method is satisfactory, but many of the records are so irregular that considerable uncertainty attaches to the graphic method. It is furthermore not adapted to routine clerical computation. For these reasons it is proposed to use the algebraic method of least squares in further study. The method gives theoretically the most probable values of the constants and is quite readily applied.

The various considerations involved in the use of equation (2) have been presented previously (*5*). It is sufficient here to say that the monthly yields of milk and fat are reduced by the formula $y_d = \frac{.4M + 15F}{L}$ where M is milk yield in pounds and F is fat yield in pounds for the month, and L is length of the month in days; y_d becomes thus the average daily energy yield in terms of 4 per cent milk in pounds for the month.[5] (One pound of 4 per cent milk = 331 large calories.) Considering the low values of k with which we shall have to deal, equation (2) is, for all practical purposes equal to

$$y_d = Ae^{-kt} \qquad (3)$$

in which $\frac{365 y_d}{12} = \frac{dy}{dt}$ and $\frac{365A}{12} = a$, provided time is computed to the *middle* of the month from which the y_d value is obtained.

Equation (3) is applied to the observed data by taking logarithms on both sides to convert it to linear form. It takes its simplest form by the use of natural logarithms:

$$\log_e y_d = \log_e A - kt \qquad (4)$$

Equation (4) is to be fitted to the 11 observations of the record by the method of least squares. Since the time origin (calving) is variable with respect to the middle of the month, it is convenient in computation to select a new time origin at the middle of the first full calendar month. Time in months from this origin we may designate T, and the time in months from calving to the middle of the first full calendar month, t'. Then $t = T + t'$. Let us designate the rate of yield at $T = 0$ as A', whence $A = A'e^{kt'}$ and $\log_e A = \log_e A' + kt'$. Equation (4) is thus equivalent to

$$\log_e y_d = \log_e A' - kT \qquad (5)$$

[4] The difference between this measure and that of Sturtevant is that the rate of decrease is computed as occurring continuously instead of monthly. The "per cent of persistency" used by Turner corresponds to $100e^{-k}$.

It should be noted that the value of a is a hypothetical quantity representing theoretical potential capacity at calving. It would perhaps be better to deal with the lactation curve following one-half or one month after calving—that is, with $ae^{-.5k}$ or ae^{-k} instead of a—thus dealing more closely with actual yields. A very simple change in equations (7)–(17) would accomplish this end.

[5] Another feature of this reduction is that it gives values lying, with few exceptions, between 10 and 100, a distinct advantage mechanically for slide rule computation.

The normal equations for the least squares determination of A' and k of (5) for 11 observations at $T=0, 1, 2, - - - 10$ are:

$$11 \log_e A' - 55k = \Sigma \log_e y_d$$
$$55 \log_e A' - 385k = \Sigma\ T \log_e y_d$$

Solving:

$$55 \log_e A' - 275k = 5\Sigma \log_e y_d$$
$$55 \log_e A' - 385k = \Sigma\ T \log_e y_d$$
$$110k = 5\Sigma \log_e y_d - \Sigma\ T \log_e y_d$$
$$10k = 5\Sigma \frac{\log_e y_d}{11} - \Sigma\ T \frac{\log_e y_d}{11}$$
$$11 \log_e A' - 55k = \Sigma \log_e y_d$$
$$\log_e A' = \Sigma \frac{\log_e y_d}{11} + 5k$$

and hence,

$$\log_e A = \Sigma \frac{\log_e y_d}{11} + 5k + kt'$$

It is clear that it is desirable to have the values $\frac{\log_e y_d}{11}$, which we may designate by v. The value v is readily computed from the original record by the use of a specially graduated slide rule as described in Figure 1. The formulae for k and A become:

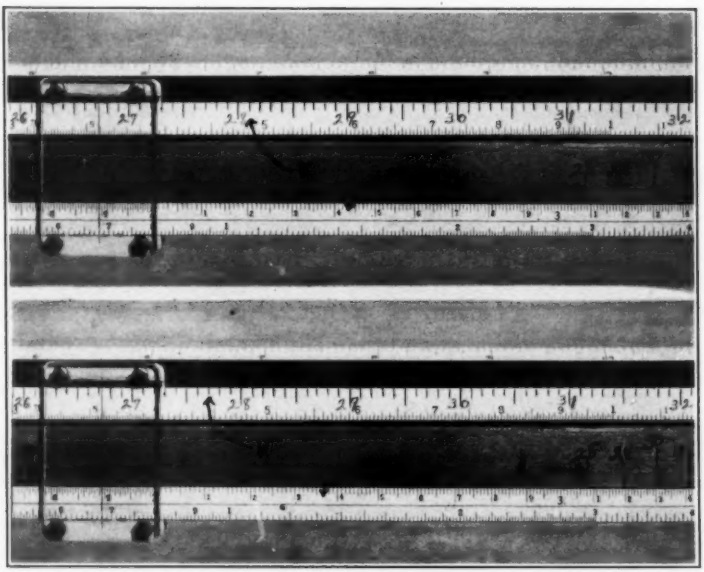

FIG. 1.—Portion of a slide rule adapted to the computation of the monthly values of v.

Example: The month record shows a 28-day month; 308 pounds of milk; 8.82 per cent fat.
Solution: Set 28 of the L scale of the slide on 308 of the M scale; set the runner on 8.82 on the f scale of slide; under the runner on the v scale read $v=0.267$ (+).
One may also read under the runner on the y_d scale, $y_d=19.0$ (−). The M and y_d scales are one and the same. Another L scale is provided at the opposite end of the slide. The f scale extends from 2.0 to 9.0. The example is chosen to condense the cut rather than as being typical.

(Formulae for use of 11 observations and natural logarithms)

$$k = .1(5\Sigma v - {}_5 Tv) \quad (6)$$

$$A = \log_e^{-1}[\Sigma v + k(5 + t')] \quad (7)$$

TABLE 1.—*Determination of the constants of the lactation curve by the method of least squares*

GUERNSEY A. R. No. 10233

Full calendar month	T= time	L= length of month	M= milk	f= fat	F= fat	$y_d = \frac{.4M+15F}{L}$	$u = \log_{10} y_d$	$v = \frac{\log_e y_d}{11}$
	Months	*Days*	*Pounds*	*Per cent*	*Pounds*	*Pounds*		
First	0	31	1,264.5	4.34	54.88	42.9	1.6325	0.342
Second	1	31	1,181.9	4.36	51.53	40.2	1.6042	.336
Third	2	30	1,179.6	4.40	51.90	41.7	1.6201	.339
Fourth	3	31	1,236.1	4.60	56.86	43.5	1.6385	.343
Fifth	4	30	1,181.6	4.47	52.82	42.2	1.6253	.340
Sixth	5	31	1,212.0	4.51	54.66	42.1	1.6243	.340
Seventh	6	31	1,205.4	4.75	57.26	43.3	1.6365	.343
Eighth	7	28	1,077.8	5.05	54.43	44.6	1.6493	.345
Ninth	8	31	1,115.8	4.81	53.67	40.4	1.6064	.336
Tenth	9	30	1,109.1	4.78	53.01	41.3	1.6160	.338
Eleventh	10	31	1,113.5	5.45	60.69	43.7	1.6405	.344
Σy_d, Σu, and Σv						465.9	17.8936	3.746
ΣTy_d, ΣTu, and ΣTv						2,337.3	89.5469	18.746
$\Sigma(T+1)\,y_d$, $\Sigma(T+1)u$, and $\Sigma(T+1)v$						2,803.2	107.4405	22.492
Rate of decrease						−.07	−.002	−.002
Initial rate of yield ($T = -1.4$)						41.9	41.9	41.9

GUERNSEY A. R. NO. 10372 [a]

First	0	31	1,609.4	4.56	73.39	56.3	1.7505	0.366
Second	1	29	1,244.1	4.96	61.71	49.1	1.6911	.354
Third	2	31	1,144.8	4.55	52.09	40.0	1.6021	.335
Fourth	3	30	1,022.9	4.52	46.24	36.8	1.5658	.328
Fifth	4	31	892.0	4.98	44.42	33.0	1.5185	.318
Sixth	5	30	856.8	4.57	30.02	23.8	1.3766	.288
Seventh	6	31	427.2	4.56	19.48	14.9	1.1732	.246
Eighth	7	31	319.9	5.19	16.60	12.2	1.0864	.227
Ninth	8	30	309.8	4.59	14.22	11.2	1.0492	.220
Tenth	9	31	288.9	5.40	15.60	11.3	1.0531	.220
Eleventh	10	30	223.7	4.67	10.45	8.2	.9138	.191
Σy_d, Σu, and Σv						296.8	14.7803	3.093
ΣTy_d, ΣTu, and ΣTv						938.6	64.2032	13.435
$\Sigma(T+1)\,y_d$, $\Sigma(T+1)u$, and $\Sigma(T+1)v$						1,235.4	78.9835	16.528
Rate of decrease						4.96	.203	.203
Initial rate of yield ($T = -1.0$)						56.7	74.5	74.5

[a] Note that No. 10372 would be dry at 11.4 months after calving ($T = 10.4$) by equation (1); by equation (2) she would be milking at the rate of 7.3 pounds of 4 per cent milk per day at that time.

Table 1, which shows the 11 full calendar-month records of two cows, illustrates the process of determining the constants of the lactation curve as just described. The data covering milk and fat yields and fat percentage are taken directly from the published record.

The values of v are computed as described in Figure 1 and recorded in column v. Σv is simply the sum of the v's and ΣTv the sum of the products Tv. $\Sigma\,(T+1)v$ is carried out to check the work. $\Sigma\,(T+1)v = \Sigma v + \Sigma Tv$. Σv and ΣTv are readily obtained on a suitable computing machine.

For No. 10233, $t' = 1.4$ and for No. 10372, $t' = 1.0$. The k constant is derived from equation (6) and the A constant from equation (7)

and a table of natural logarithms. Having given the record itself, the only figures necessary to record are those given in the v column of Table 1. The whole operation thus becomes engagingly simple.

It is, of course, unnecessary to compute A if the measure of persistency is all that is desired. But it appears (3) that for equitable comparisons of the k's it is necessary to know the values of the A's for the purpose of correction.

Where conception occurs early in the record, pregnancy may introduce a disturbing factor in the later observations. The decrease in rate of yield due to pregnancy is not large and may be allowed for as follows: If at the end of the last month used the calf is carried less than 183 days no correction need be made. Where the calf is carried 183 to 213 days as above, correct the last observation by multiplying by 1.02, that is, *add* 0.002 to the last observed v. Where the calf is carried 214 to 244 days, correct the next to last observation as above, and correct the last observation by the factor 1.06, that is, *add* 0.005 to the last v. These corrections are based on the effect of pregnancy as previously given. (*5*)

Where pregnancy is more than 244 days advanced as above, it may be desirable to work with nine observations or with seven, as the case requires. Corrections to the last one or two v's are made as indicated above.

The normal equations using nine observations are:

$$9 \log_e A' - 36k = \Sigma \log_e y_d$$

$$36 \log_e A' - 204k = \Sigma T \log_e y_d$$

From which we derive:

(Formulae for use of nine observations and natural logarithms.)

$$k = .183(4\Sigma v - \Sigma Tv) \quad (8)$$

$$A = \log_e^{-1} [1.222\Sigma v + k(4 + t'')] \quad (9)$$

The normal equations using seven observations are:

$$7 \log_e A' - 21k = \Sigma \log_e y_d$$

$$21 \log_e A' - 91k = \Sigma T \log_e y_d$$

From which we derive:

(Formulae for use of seven observations and natural logarithms.)

$$k = .393(3\Sigma v - \Sigma Tv) \quad (10)$$

$$A = \log_e^{-1} [1.571\Sigma v + k(3 + t')] \quad (11)$$

The majority (about 80 per cent) of the Guernsey records may be handled with equations (6) and (7). The value v is not particularly useful in connection with equations (8), (9), (10), and (11), but it still affords as easy a solution as may be had; and its retention is justified in order to utilize the already established device of Figure 1.

If it is desired to work directly with the milk or fat yields, or if the equation is to be fitted to only a small number of records, it may be preferable to use common logarithms instead of natural logarithms.

The data necessary to record and the final results are given as examples in column u of Table 1. The following formulae in which $u = \log_{10} y_d$, give results identical with those above.[6] To correct for pregnancy add 0.0086 and 0.0253 to u in the same manner as 0.002 and 0.005, respectively, to v above.

(Formulae for use of 11 observations and common logarithms.)

$$k = .0209\ (5\Sigma u - \Sigma Tu) \quad (12)$$

$$A = \log_{10}{}^{-1}[.09091\Sigma u + .4343k\ (5 + t')] \quad (13)$$

(Formulae for use of 9 observations and common logarithms.)

$$k = .0384\ (4\Sigma u - \Sigma Tu) \quad (14)$$

$$A = \log_{10}{}^{-1}\ [.11111\Sigma u + .4343k\ (4 + t')] \quad (15)$$

(Formulae for use of observations and common logarithms.)

$$k = .0822\ (3\Sigma u - \Sigma Tu) \quad (16)$$

$$A = \log_{10}{}^{-1}\ [.14286\Sigma u + .4343k\ (3 + t')] \quad (17)$$

Another method of determining the k constant deserves consideration. From equation (2) the rate of yield at one month after calving is ae^{-k} and the yield for 12 months is $a\,\frac{1 - e^{-12k}}{k}$. Accordingly, the ratio of the former to the latter is $\frac{ae^{-k}}{a\,\frac{1 - e^{-12k}}{k}} = \frac{k}{e^{k} - e^{-11k}}$.

It is apparent that the a's cancel and any given value of k results in a certain definite ratio. Conversely, if the ratio is given, the value of k may be computed. We have given the 12 months' yield. If we let the yield of the first full calendar month represent the rate of yield one month after the record starts (assuming that the first of that month is one-half month after the beginning of the record), we may determine the required ratio.

The theoretical ratios corresponding to various values of k are as follows:

k	−0.04	−0.03	−0.02	−0.01	0	0.01	0.02	0.03	0.04	0.05
Ratio [a]	2,222	2,345	2,473	2,605	2,740	2,879	3,020	3,166	3,315	3,466
Ratio [b]	2,312	2,417	2,523	2,631	2,740	2,850	2,960	3,072	3,185	3,297
k	0.06	0.07	0.08	0.09	0.10	0.11	0.12	0.13	0.14	0.15
Ratio [a]	3,619	3,776	3,934	4,095	4,257	4,421	4,585	4,751	4,919	5,084
Ratio [b]	3,408	3 521	3 632	3,743	3,852	3,960	4,067	4,172	4,275	4,376
k	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25
Ratio [a]	5,252	5,420	5,587	5,754	5,921	6,086	6,250	6,414	6,575	6,734
Ratio [b]	4,476	4,572	4,666	4,757	4,847	4,933	5,015	5,097	5,173	5,247

[a] $\frac{12}{365} \times \frac{k}{e^{k} - e^{-11k}} \times 10^{6}$. This ratio is based on the use of the first full calendar month's yield per day, assuming the middle of this month to be one month after the beginning of the record.

[b] $\frac{12}{365} \times \frac{k}{e^{2k} - e^{-10k}} \times 10^{6}$. This ratio is based on the use of the second full calendar month's yield per day, assuming the middle of this month to be two months after the beginning of the record.

It should be observed that the relation between the k's and the ratios is not linear. Persistency as measured by the k's is therefore not directly comparable with persistency as measured by the ratios.

[6] It is of course easier to deal directly with the calendar month milk or fat yields, in which case let $u = \log_{10} M$ and $\log_{10} F$, respectively, and $A = a$, in equations (12) to (17). As Turner has pointed out, it is necessary to distinguish between persistency in *milk* yield and persistency in *fat* yield—the decrease in rate of fat yield being ordinarily less rapid than the decrease in rate of milk yield. Persistency in *energy* yield is intermediate (*5, fig. 1*), and takes account of the variability between individual cows in the change of fat percentage with advance in lactation.

The estimation of k by this method is very rapidly carried out by the use of an appropriately graduated slide rule. The slide carries the L and f scales as shown in Figure 1 and in addition, a regular logarithmic scale matching the logarithmic M scale. A k scale replaces the v scale of Figure 1. The computation requires two settings of the slide and one setting of the runner, the value of k being read opposite the unity graduation of the logarithmic scale of the slide.

By entirely similar methods the ratio of the second month to the year may be used in place of the ratio of the first month. The ratios for the second month are also given in the tabulation immediately preceding

The value of A is given by the formula $A=\log_e^{-1}(\log_e y_d+t')$ where y_d is determined from the first month and t' is time in months from calving to the middle of the first month. The estimations assume that pregnancy is not a factor.

The constants of the straight line equation (1) are determined thus

$$b=.0091(5\Sigma y_d-\Sigma T y_d) \quad (18)$$

$$C=.0909\Sigma y_d+b(5+t') \quad (19)$$

where 11 observations are used and pregnancy is not a factor to be considered. Examples of the application are given in the y_d column of Table 1.

COMPARISON OF PERSISTENCY MEASURES

From the Guernsey records previously used (*3*) in which k of equation (2) was determined graphically, 100 were selected at random, except that only records were taken in which not more than 30 days elapsed from calving to the first of the first full month. For the purpose of comparison the value of k in these 100 records has been computed by least squares and the two ratio methods; also the value of b in equation (1) has been computed by (18). In none of these records was pregnancy far enough advanced to require a correction.

The values of k and b have been correlated with the values of k by least squares, giving the constants set forth in Table 2. The mean values of k as determined graphically and by the ratio methods do not differ significantly from the mean as determined by the method of least squares. A high degree of variability as measured by the coefficient of variability is evident throughout.

TABLE 2.—*Statistical constants of persistency of lactation as determined by different methods*

Measure of persistency	Mean	Standard deviation	Coefficient of variability	Relation to k by least squares—coefficient of correlation
k by least squares	0.0382±0.0020	0.0300±0.0141	78.52	
k graphically	.0398± .0021	.0311± .0148	77.94	0.956±0.006
k from first month and year	.0377± .0021	.0304± .0148	80.53	.866± .017
k from second month and year	.0415± .0024	.0350± .0170	84.34	.902± .013
b by least squares	1.118 ± .058	.857 ± .041	76.66	.921± .010

The mean value of b is, of course, much greater than that of k, but it is of interest to note that the coefficient of variability of b is nearly the same as that of k. The coefficients of correlation show that taking k by the method of least squares as the standard, k by the graphic method gives the next best result; b by least squares next; followed by the second month ratio. The first month ratio is least closely related.

DISCUSSION

If we accept the monthly observations as each of equal reliability and accept also equation (2) as a proper description of the lactation curve, then k as determined by the method of least squares should be the most probable persistency value for any given record. The k values obtained graphically correspond reasonably well; $r=0.956$. While the graphic method possesses some advantage in speed, the algebraic method has the advantage of being an exact process and adapted to routine computation.

In the graphic treatment 8.5 per cent of the records were rejected because of extreme irregularities (3). The fact that the algebraic process leads to a perfectly definite final result for these records as well as the others does not make that final result of perfectly definite significance. For that matter and from the same point of view, the definite figure of the 12 months' yield may be lacking in definite significance. The value of k determined by least squares would seem to afford a measure of persistency at least as definite in significance as the year's yield itself.

The estimation of k by the ratio method has a great advantage in point of speed, and it was largely to test its reliability that the present comparisons were made. Apparently it is better to use the second month rather than the first, although it is doubtful if the difference is significant. The reliability of this rapid method of estimating k may be tested by correlating the k's as determined from the first and second months. The correlation surface given in Table 3 shows $r=0.792$. There would seem to be some question, therefore, as to the trustworthiness of this method of estimating k.[7] This is unfortunate, since the method is a short and easy one.

The ratio method used by Sanders (*7*) and Turner (*9*), namely $\frac{\text{total yield}}{\text{maximum rate of yield}}$, fails to distinguish the ascending lactation curve from the more usual descending curve. Inasmuch as about 5 per cent of the Guernsey records show an ascending lactation curve, it is evident that this feature should be distinguished. Furthermore, as will be sufficiently evident from the ratios given above, the use of a ratio of this kind as a consistent measure of persistency must take the short time yield at a definite stage of the longer time period.

The use of equation (1) gives a measure of persistency which is apparently in close keeping with that of the least squares k of equation (2), $r=0.921$. So far as the writer can see, there is no a priori reason why b of (1) should not be used as a measure of persistency. Nevertheless, it appears more reasonable to express the rate of decrease as proportional to the level of yield.

[7] The present treatment takes account of the length of the calendar month. Failure so to do would, of course, add to the uncertainty of the result.

TABLE 3.—*Correlation of the k's computed from the ratio of the first month's yield to the year's yield and the ratio of the second month's yield to the year's yield* [a]

$k\times10^3$ from second month and year—class mid-points	$k\times10^3$ from first month and year—class mid-points														Total
	−25	−15	−5	5	15	25	35	45	55	65	75	85	95	105	
−25		1													1
−15			1	1	1										3
−5	1	1	2	1		1		1							7
5	1		1	3		1		1							7
15	1		1	1	1	1		1	1						7
25			1	1	2	3	6	2	1						16
35				1	2	2	5	2		1					13
45				1	1	2	1	4	3						12
55							1	1	4	1	1		1		9
65						1		1	1	3	1				7
75					1			2	2					1	6
85							1		1			1			3
95												1			1
105													2		2
115											1	2		1	4
125															0
135															0
145													1		1
155														1	1
Total	3	2	6	9	8	11	14	15	13	5	3	4	4	3	100

[a] $r=0.792\pm0.025$. See Table 2 for other constants.

The lactation curve is distinctly nonlinear, but only slightly so, [cf. numerous figures (*1*, *5*)]. Indirect statistical evidence that such is the case is afforded by the correlation between rate of yield and yield for the year. By (1) it is evident from the simple geometry of the equation that the area from $t=0$ to $t=12$ would be entirely determined at $t=6$, regardless of the slope of the curve (granting that $C>12b$). We should accordingly expect the highest correlation between rate of yield and year's yield when the rate of yield is taken at $t=6$. The observed correlations show the highest value somewhat earlier than this. On the basis of the descending exponential curve, this is to be expected.

Another and much more important distinction between the two types of equations as a measure of persistency, may be brought out by an illustration. McCandlish et al. (*6*, *chart 2*) have presented graphically the lactation curves of purebred dairy cows and scrub cows in the Iowa Station herd. Fitting equations (1) and (2) to the monthly milk yields [8] by the method of least squares gives the following:

	Purebred cows	Scrub cows
Equation (1),	$\frac{dy}{dt}=1055-70.9t$	$\frac{dy}{dt}=629-55.9t$
Equation (2),	$\frac{dy}{dt}=1169e^{-.114t}$	$\frac{dy}{dt}=848e^{-.213t}$
Rate of decrease by (1)	70. 9	55. 9
Rate of decrease by (2)	. 114	. 213

By the linear equation the scrub cows are considerably more persistent milkers; i. e., show a smaller rate of decrease than the pure-

[8] Professor Kildee has kindly furnished the numerical data of monthly (30-day) milk yields from which the present equations are derived.

breds. By the exponential equation the purebreds are nearly twice as persistent as the scrubs. Are the scrubs or the purebreds the more persistent?

Critical study of persistency and of the lactation curve in general has not yet been carried to a point that affords very complete knowledge of the subject. The available data should be utilized to the fullest extent for the purpose of obtaining a correct understanding of the characteristics, heritable and otherwise, of the lactation curve.

The official records as now published by breed organizations do not give any direct information as to persistency. In particular, the year's yield is an exceedingly poor index of persistency. Contrary to general opinion, the seven-day record early in the lactation far excells the year record in this respect. One object of securing a satisfactory numerical description of the lactation curve (not necessarily the easiest of computation) is the possibility that such a description offers of presenting the essential facts in condensed form and thus within the means of publication for individual records.

SUMMARY

Several methods for measuring persistency of lactation are reviewed. The measure preferred by the writer is the constant k in the equation $\frac{dy}{dt}=ae^{-kt}$, in which y is yield, t is time, and $\frac{dy}{dt}$ is the rate of yield. The constant k measures the rate of decrease in rate of yield as proportional to the rate of yield. It is thus an inverse measure of persistency. Formulae for fitting the equation by least squares are given in simplified form for the special cases of 7, 9, and 11 observed values.

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THE VITAMIN C CONTENT OF FRESH AND CANNED PEAR[1]

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INTRODUCTION

It has become a well-established fact that the water-soluble vitamin C is necessary not only as a protection from scurvy but also in normal metabolism and in the maintenance of the defense of the body against the attacks of infectious diseases. Since vitamin C is necessary in the adequate diet, it is expedient that a knowledge of foods which can be used to supply this factor be acquired. Little is known in regard to the antiscorbutic value of some of the more common fruits which are grown in this section of the country and which are abundant in the local markets. This investigation was made in order to determine whether or not pears which were grown locally could be used as a source of vitamin C, and therefore substituted for citrus fruits, which are purchased for their antiscorbutic value.

Data are not available on the vitamin C content of pear, but investigations have been conducted on other fruits which may be grown in this region. Kohman, Eddy, and Carlsson (*7*),[3] in their work with apples, indicate that vitamin C is destroyed by heat and oxidation in the processes of cooking and canning if no preliminary treatment has been given. They found that when raw apples were held in cold storage a marked deterioration occurred in the vitamin C content, although storage seemed to make no difference after the fruit was canned. Givens, McClugage, and Van Horne (*2*) found 10 gm. of raw apples to be a minimum protective dose against scurvy in guinea pigs. They reported that exposure to temperatures necessary for drying or canning caused a marked loss in the antiscorbutic factor. The minimum protective dose of raw peach was found by Kohman, Eddy, and Carlsson (*8*) to be 5 gm. daily. A daily dose of 20 gm. was necessary when the fruit was cooked in a covered kettle for 15 minutes. They found that the commercially canned peach had between four and five times as much vitamin C as fruit cooked in a covered kettle.

The present paper deals with the determination of the minimum of fresh pear required to prevent scurvy in guinea pigs. This determination was then used as a basis for estimating the loss in the antiscorbutic value which occurs in the pear when preserved according to the open-kettle and cold-pack methods of home canning.

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[3] Reference is made by number (italic) to "Literature cited," p. 392.

METHODS

The vitamin C determinations were made in the usual manner on guinea pigs. The procedure of Sherman, LaMer, and Campbell (*12*) was closely followed. Cages, in which the animals were confined singly, were cleaned on alternate days and sterilized weekly. During the entire period the animals were weighed three times a week and all abnormal symptoms noted.

The basal diet recommended by the above-named authors is as follows:

	Per cent
Oats, sound whole grain, ground	59
Skimmed milk powder, heated for two hours at 110° C	30
Butter fat, filtered and freshly prepared	10
Sodium chloride	1
	100

This diet was slightly modified by substituting cod-liver oil for 2 per cent of the butterfat.

Kieffer pears of sound quality and purchased on the local market in September were used. In February the supply of fresh pears was exhausted, and a new lot of California pears was purchased to finish the experiment. This product was fed raw until April, the animals showing the same growth as had been made while they were fed on the home-grown product. Some of the raw pears were kept in a fruit-storage room at a temperature of 40° F. They were wrapped separately in paper so as not to come in contact with each other, and every two weeks they were sorted and the spoiled ones removed.

The ripest of the Kieffer pears were canned according to the following methods of home preservation:

1. *The open-kettle method.*—The fruit was peeled, cored, and halved. In an open kettle were placed 430 gms. of raw pear, 100 gms. of sugar, and a minimum amount of distilled water. When the boiling point was reached, the fruit was allowed to boil for 15 minutes and immediately sealed in sterile pint jars.

2. *The cold-pack method.*—The fruit was prepared as in the open-kettle method. The same amounts of fruit and sugar were placed in sterilized jars, and enough distilled water was added to fill the jars. They were sealed and processed for 20 minutes after the temperature of the water surrounding them reached the boiling point. In the household the jars are sealed after the processing, but in this experiment the order was reversed to avoid possible loss of product.

With a known amount of fruit, sugar, and water in each can, it was possible to calculate, in terms of raw pear, the actual amount of canned pear fed. The amount of pear given to each animal was calculated on the basis of the amount that would have been fed to a 300-gm. guinea pig, as it was thought that by so doing more uniform results would be obtained. In feeding the canned pear it was found that the entire amount given each day was not always consumed, perhaps because of the sugar, in the pear. It was, necessary therefore, to calculate the amount wasted, which may have introduced an element of error into the results.

The raw pears were allowed to ripen in storage, and feeding was started in November. The animals were given 5, 10, 15, 20, 25, 30, or 35 gm. amounts of raw pear, or 10, 15, 20, 25, 30, or 35 gm. amounts of canned pear. Fifty-five animals, 53 males and 2 females, were fed. Negative control animals were given only the

basal diet in order to make sure that they received no vitamin C. Positive control animals were given the basal diet and 5 c. c. of tomato juice.

Autopsies were performed in all cases soon after death occurred. Bacteriological cultures were made to determine whether or not infections existed. A study of the gross microscopic pathology was made according to Hess (*5*). The histological method used was that suggested in the study made by Wolbach and Howe (*13*).

RESULTS

The symptomatology of scurvy which was found in the present experiment was similar to that described by Jackson and Moore (*6*), Cohen and Mendel (*1*), Hart, Steenbock, and Smith (*3*), Hess (*5*), and others. The first indication was lameness of the hind legs, followed by soreness and swelling in the joints and muscles and an

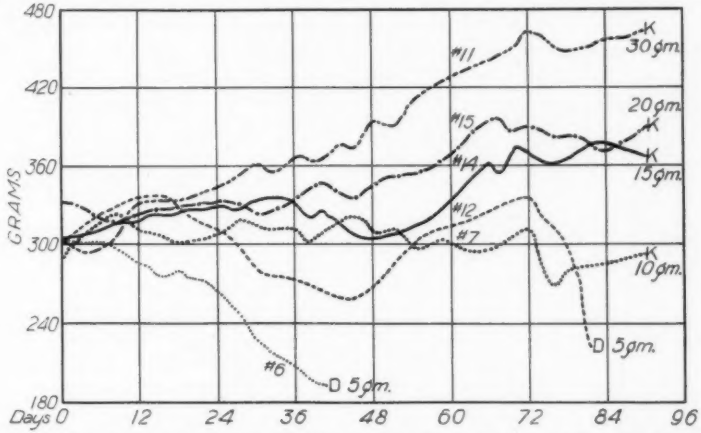

FIG. 1.—Growth curves of guinea pigs receiving from 5 to 30 gm. of raw pear daily. The growth curves of animals not surviving the 90-day experimentation period are marked "D"; those of animals killed at the expiration of the 90 days are marked "K"

increasing inactivity, which finally developed into an apparent paralysis of those parts. There was an increased flabbiness of the muscles and a general loss in body weight, resulting in emaciation. The autopsies showed hemorrhage in the muscles, particularly in the tissue surrounding the femur and the costochondral junctions. In some cases intestinal hemorrhage, preceded by bloody diarrhea, was noticed, while in others there was hematuria. Some congestion was found in the stomach and bladder, and in three cases an ulcerated condition in these organs. Contrary to the findings of Jackson and Moore (*6*), almost no hemorrhagic condition was observed in the gums. The teeth became yellow with the onset of the disease. Different degrees of looseness were found in the lower molars, and in some instances these could be removed easily with forceps. In no case were the incisors loose, and some animals had no loose teeth at all. The costochondral junctions, particularly of the middle ribs, were found to have the characteristic beading of scurvy. The spleen and lymph nodes were enlarged in two instances, a condition described by Jackson and Moore (*6*).

A study of the microscopic pathology was made according to Hess (*5*), only gross changes in the tissues being noted. The study of the costochondral junctions showed disorganization of the cells.

As is indicated in Table 1, two animals which were given 5 gm. amounts of raw pear did not survive the 90-day experimental period. Guinea pig 6, on 5 gm. of raw pear, died at the end of 40 days (fig. 1), and had only very slight symptoms of scurvy. There was a slight hemorrhage in the costochondral junction, some fatty degeneration of the liver, and slight congestion in the lungs. These symptoms might indicate reasons for death other than scurvy, although the bacterial cultures showed no pathogenic organisms. Guinea pig 12, on 5 gm. of raw pear, showed some hemorrhage in the stomach, but the other organs appeared normal. The bones were somewhat brittle, and the lymph nodes had become enlarged. The animals fed 10 gm. of raw pear gave no indications of scurvy during life, and the autopsies revealed no sign of a vitamin C deficiency. The only indication was a negligible loss in body weight. The 15 gm. amounts gave good protection to the animals, as is shown by the fact that in most cases there was some gain in body weight. The other animals on varying amounts of raw pear made varying gains in weight. (Table 1 and fig 1.) The unexpected gain made by guinea pig 17 on 30 grams of raw pear may have been due not only to the large amount of fruit given but also to the fact that it was a larger animal at the beginning of the experiment.

TABLE 1.—*Protocols of experimental animals fed the basal diet and raw pear*

Animal No.	Amount of pear eaten daily	Initial weight of animal	Final weight of animal	Gain or loss in weight	Duration of experiment
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Days*
6	5	302	187	−115	40
12	5	302	216	−86	81
7	10	308	290	−18	90
13	10	339	333	−6	90
9	15	295	281	−14	90
8	15	295	394	+99	90
14	15	303	363	+60	90
19	15	305	328	+23	90
15	20	332	383	+51	90
20	20	359	478	+119	90
10	25	308	334	+26	90
16	25	300	356	+56	90
11	30	303	458	+155	90
17	30	336	575	+239	90
18	35	305	437	+132	90

The average survival period for the animals fed open-kettle pear was 25.2 days. These animals were given large quantities of the canned fruit, but the entire amounts were not always eaten by every guinea pig. As is indicated in Table 2, it was found that 8.9 grams was the smallest amount and 19.1 grams was the largest amount of pear eaten daily. Table 2 also shows that characteristic scurvy lesions were found in the animals fed on open-kettle pear. The symbols x, xx, and xxx are used to denote increasingly marked findings.

TABLE 2.—*Protocols of experimental animals fed the basal diet and open-kettle pear*

Animal No.	Amount of pear eaten daily	Initial weight of animal	Final weight of animal	Loss in weight	Duration of experiment	Degree of malnutrition,[a] as indicated by—		
						Teeth	Bones and ribs	Hemorrhage
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Days*			
49	8.9	326	187	139	32	xx[b]	xx	x
50	10.9	320	214	106	27	xx	x	xx
52	12.9	297	248	49	19	xx	x	xx
59	15.6	297	214	83	15			x
57	17.1	220	195	25	19	xx	x	xxx
53	17.6	306	280	26	27	xx	xx	xx
58	18.1	299	241	58	22		x	xx
54	18.3	304	295	9	24	x	x	xx
56	18.3	320	173	147	30	xx	x	xxx
51	19.0	305	176	129	38	xxx	xx	xx
60	19.1	304	265	39	25	x	xx	xx

[a] Determined by post-mortem examination.
[b] The symbols x, xx, and xxx, indicate the relative severity of the condition found.

Figure 2 shows the growth curves of the guinea pig eating the smallest amount and of the one eating approximately the largest amount daily, and it can readily be seen that the larger amount gives very little more protection than does the smaller amount. In fact, the animal eating the largest daily amount (No. 60) lived only 25 days, which is slightly below the average for the group. In no case did any animal fed open-kettle pear live more than 38 days—an interval only a little longer than the average expectation of life of the animals on the basal diet alone, which, according to Sherman, La Mer, and Campbell (*12*), is 26 to 34 days. The figures for this series fall within those limits. Figure 3 shows the growth curves of three animals—one eating the smallest amount of cold-pack pear daily, one an approximately medium amount, and one the largest amount.

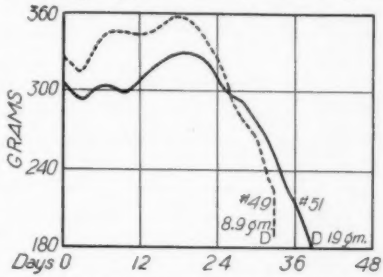

FIG. 2.—Growth curves of two guinea pigs, one eating 8.9 gm. and the other 19 gm. of open-kettle pear daily. The growth curves are marked "D," indicating that neither animal survived the full experimentation period of 90 days

Number of grams of pear eaten daily	Average survival period, in days
5–9	29. 5
9–13	34. 4
13–17	38. 6
17–21	38.
21–25	[a] 50.

[a] One guinea pig.

In no case did an animal survive the experimental period and all the guinea pigs showed some symptoms of scurvy in the post-mortem examinations. The extent of these symptoms is indicated in Table 3. The autopsy findings showed that severe symptoms did not always accompany early death.

TABLE 3.—*Protocols of experimental animals fed basal diet and cold-pack pear*

Animal No.	Amount of pear eaten daily	Initial weight of animal	Final weight of animal	Loss in weight	Duration of experiment	Degree of malnutrition[a] as indicated by—		
						Teeth	Bones and ribs	Hemorrhage
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Days*			
42	6.80	356	214	142	25	x x[b]		
43	7.18	358	210	148	32	x	x x x	x
31	8.00	317	189	128	33	x x x	x x x	x x
44	8.89	363	227	136	28	x	x x	x x
46	9.87	346	185	161	37	x x x	x x	x x
47	10.00	348	183	165	46	x x x	x	x x
37	10.90	362	252	110	22	x	x x	x
38	11.36	388	210	178	33	x	x	x
41	12.00	302	166	136	25	x x	x x	x x
35	12.00	357	194	163	37	x x x	x x	x x
48	12.20	357	192	165	41	x x x	x x x	x x
25	13.00	329	195	134	33	x x	x x	x x
26	14.30	322	206	116	40		x x x	x x
24	14.34	285	164	121	46		x	x x
36	14.60	320	193	127	44	x x	x x	x x
27	15.60	356	223	133	30		x x x	x x x
28	17.00	340	197	143	27		x x x	x x x
40	20.40	377	219	158	49	x x x	x x	x
29	22.50	291	205	86	50		x x	x x

[a] Determined by post-mortem examination.

[b] The symbols x, x x, and x x x indicate the relative severity of the condition found.

The duration of life of the guinea pigs fed on the cold-pack pear varied from 22 to 50 days and averaged 35.7 days. The animals that lived longer were, as a rule, those that ate the larger amounts of pear. As only a comparatively few cases are under consideration, they show wide variation; averages are used, therefore, to show the general trend of the results.

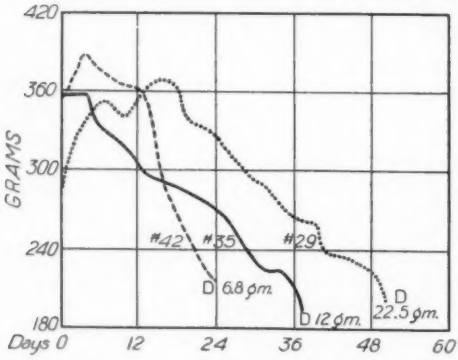

FIG. 3.—Growth curves of three guinea pigs, one eating 6.8 gm., another 15 gm., and the third 22.5 gm., of cold-pack pear daily. The curves are marked "D," indicating that the animals failed to survive the full experiment period of 90 days

DISCUSSION

It has been indicated in this experiment that the minimum protective dose of raw pear is a little more than 10 gm. and less than 15 gm. It was not possible to find amounts of canned pear sufficient to give protection from scurvy. The vitamin C content of open-kettle pear must be practically all destroyed, as the average survival period was no greater than the expected survival period of animals on the basal diet alone, while the antiscorbutic value of cold-pack pear is not so completely destroyed, since the larger amounts usually increased the survival period slightly. Factors entering into the destruction of vitamin C in the home processes of canning pear are probably heat and oxidation.

The P_H of the canned pear was 4, as determined by the colorimetric method, the bromphenol blue, 0.02 per cent aqueous solution being used according to Medalia (*10*). Harvey (*4*), in his determination with the standard hydrogen electrode equipment, found the P_H of pears purchased in the open market to be 4.2. LaMer, Campbell, and Sherman (*9*) found the P_H of tomatoes to be 4.3. According to their results less destruction of the vitamin C content in the canning of pears might have been anticipated.

The antiscorbutic value of raw pear might be considered about 25 per cent of the potency of orange juice or of tomato juice, since the minimum protective dose of pear is about 12 gms. as compared to 3 c. c. of tomato juice which, according to Sherman (*11*), is necessary to give complete protection.

The relative richness in vitamin C of certain fruits as given by Sherman (*11*) is as follows:

Orange juice	100
Lemon juice	100
Pineapple, fresh, raw	70
Lime juice	25
Banana	20–40
Apples, raw	10–20

If orange juice is taken as 100, raw pear would have a potency of 25.

SUMMARY AND CONCLUSIONS

Observations made in this study lead to the following conclusions:

The minimum protective dose of raw pear for guinea pigs is between 10 and 15 gms. The animals on 10 gms. of raw pear daily gave no indications of scurvy during life, and the autopsies revealed no sign of a vitamin C deficiency. The only indication was a negligible loss in body weight. The 15-gm. amounts fed daily gave good protection to the animals and some gain in body weight.

The potency of raw pear is estimated as 25 if orange juice is regarded as 100.

The antiscorbutic factor in pear is destroyed in the open-kettle method of home canning. This was shown by the fact that the animals fed open-kettle pear lived on the average no longer than the expected survival period when the basal diet alone was given.

The antiscorbutic factor in pear is not completely destroyed by the cold-pack method of home canning. In no case, however, did the animals fed cold-pack pear survive the experimental period of 90 days.

The storage of fresh pear seemed to make no difference in the antiscorbutic content, since the animals fed fresh pear made the same rate of gain during the later part of the experimental period as they made earlier in the year when they were fed fresh pear that had not been stored.

The California pear was no more potent than the home-grown fruit, as was evidenced by the fact that no marked change in the weight curves of the animals occurred when California pear was substituted for home-grown pear.

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